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STUDIES OF 17-HYDROXYCORTICOSTEROIDS. X. URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS IN PATIENTS WITH RHEUMATIC FEVER*

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ONE approach to the evaluation of adrenocortical function is the measurement of urinary excretion of adrenocortical steroids or their metabolites. Until recently the methods used for such evaluation have included biologic assay technics and chemical determinations of urinary corticoids and 17-ketosteroids. These technics are of limited value because they lack either specificity or sensitivity. Several investigators (1–4) recently have introduced technics for the determination of urinary 17-hydroxycorticosteroids (17-OHCS) employing the Porter-Silber reaction (5), which is specific for the 17, 21-dihydroxy-20-keto configuration. The 17-hydroxycorticosteroids are a group of adrenal steroids which includes compounds F (hydrocortisone), E (cortisone), and S. Since compound F has been

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demonstrated to be the principal adrenocortical hormone in man (6, 7), determinations of urinary 17-OHCS might be expected to reflect more accurately the function of the adrenal cortex than do the older, less specific determinations of urinary steroids.

It has been demonstrated that patients with rheumatic fever have abnormal concentrations of circulating 17-OHCS (8), with elevated plasma levels of these steroids during the "early acute" phase of the disease and subnormal levels during the "well-established active" and inactive phases. These concentrations are altered by therapy (9). Thus, the plasma level is continuously high during treatment with ACTH-gel or intramuscular cortisone, fluctuates widely during oral cortisone or lyophilized ACTH intramuscular therapy, is unaffected during bed rest, and is depressed to low values during treatment with salicylates.

The present report is concerned with the urinary excretion of 17-OHCS by patients with rheumatic fever before, during, and after various types of therapy. Excretion values of 17-OHCS in a control group of children are presented and compared with those observed in children with rheumatic fever, and in a control group of normal adults.

MATERIALS AND METHODS

The subjects of this study included a control group of 26 children ranging in age from 6 to 15 years, a control group of 23 young adult males, and 30 children with active rheumatic fever ranging in age from 6 to 15 years. The diagnosis of rheumatic fever was established according to the criteria of Jones (10), with the modifications introduced by Denny and co-workers (11). Serial studies were made in the rheumatic patients before, during, and after the period of therapy. The forms of treatment used included cortisone[1] given orally, ACTH-gel,[2] salicylates, and bed rest alone. Details of dosage schedules are reported elsewhere (12); but the doses used in this study are indicated in Table 3 and in the figures which present the data concerning illustrative patients. The 24-hour urinary 17-OHCS excretions were determined by the method of Glenn and Nelson (2).

OBSERVATIONS

In Table 1 are shown the data concerning normal urinary 17-OHCS excretion values in a 24-hour period for control groups of children and adults. The mean value for the young adult males was 5.6 ± 0.63 mg. per twenty-four hours, which is in general agreement with data reported from other

[1] Cortone®, supplied through the courtesy of Merck and Co., Inc. Rahway, N. J.

[2] ACTHARGEL®, supplied through the courtesy of the Armour Laboratories, Chicago.

TABLE 1. 24-HOUR URINARY 17-OHCS EXCRETION IN ADULTS *vs.* CHILDREN

	Control adults (males)	Control children (6–15 yrs. of age)
No. determinations	24	38
No. subjects	23	26
17-OHCS excretion		
Mean (mg./day)	5.6	2.2
SEM	±0.63	±0.27
p	<0.01	

laboratories (1, 2, 4). In contrast, the mean value for children from 6 to 15 years of age was 2.2±0.27 mg. per twenty-four hours. It may be seen that a significant difference ($p < 0.01$) exists between the children and the adults, the former having a value less than half that in the adults.

In Table 2 the urinary 17-OHCS excretion in the control group of children is compared with that in children with untreated active rheumatic fever. For the latter group a mean value of 2.0±0.12 mg. per twenty-four hours was obtained, which is essentially the same as that found in the control group. Although plasma concentrations of 17-OHCS decrease progressively during the acute phase of rheumatic fever (8), the urinary excretion of these steroids did not change appreciably during this period.

Data pertaining to the 17-OHCS excretion by these patients during the period of hormone treatment for rheumatic fever are presented in Table 3. The therapy groups represented are those receiving cortisone or ACTH-gel. The steroid excretion is presented in relation to the size of the dose administered. During the period of administration of cortisone there was a large increase in the mean 17-OHCS excretion compared with that ob-

TABLE 2. URINARY 17-OHCS EXCRETION IN CHILDREN WITH RHEUMATIC FEVER

	Control children	Children with untreated active R.F.
No. determinations	38	92
No. subjects	26	30
17-OHCS excretion		
Mean (mg./day)	2.2	2.0
SEM	±0.27	±0.12
p	>.5	

TABLE 3. URINARY 17-OHCS EXCRETION DURING HORMONE THERAPY IN PATIENTS WITH RHEUMATIC FEVER

	Cortisone (mg./day)				ACTH-gel (I.U./day)				
Dosage	250–300	150–200	80–100	25–50	150	90–110	70–80	40–60	10–25
No. determinations	24	71	9	6	2	19	32	25	17
No. patients	2	8	4	3	2	4	6	5	5
17-OHCS excretion									
Range (mg./day)	2–119	0–111	0–59	0–9	42–174	13–281	4–288	3–121	0–53
Mean (mg./day)	61	28	20	3.7	108	80	50	36	13
SEM	±7.5	±3.2	±5.8	±1.4	—	±16.4	±11.1	±5.7	±4.1

served in untreated patients. Following reductions in the dose of cortisone these increased steroid excretions became progressively less. When the dose was reduced to 25–50 mg. per day, the range of 17-OHCS excretion values was 0–9 mg. per twenty-four hours and the mean was 3.7 mg., a value comparable to that in the untreated rheumatic patients. A similar relationship obtained between the dose of ACTH-gel and steroid excretion.

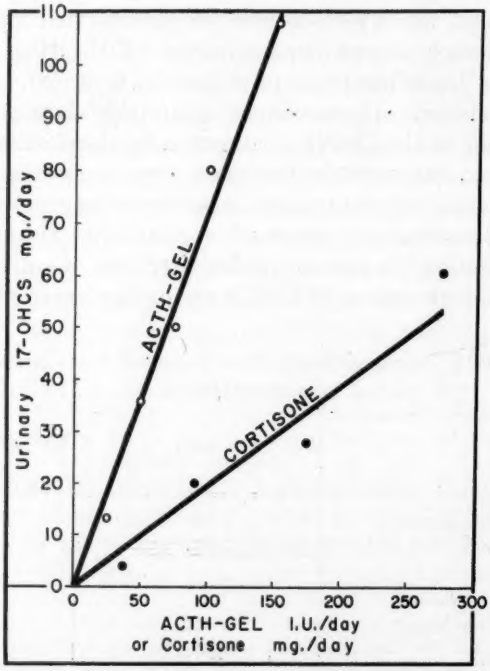

FIG. 1. Urinary 17-OHCS excretion *vs.* dose of cortisone or of ACTH-gel.

However, the mean increases in urinary excretion which occurred in response to ACTH-gel were consistently much greater than those which occurred in response to cortisone. Whereas the mean steroid excretion values were approximately normal in patients receiving 25–50 mg. of cortisone per day, they were distinctly elevated in those receiving 10–25 I.U. of ACTH-gel. From the data shown in Table 3 it would appear that 1 I.U. of ACTH-gel is roughly equivalent to 3–4 mg. of cortisone in terms of the induced increase in urinary 17-OHCS excretion.

Figure 1 presents graphically a comparison of the effects of ACTH-gel

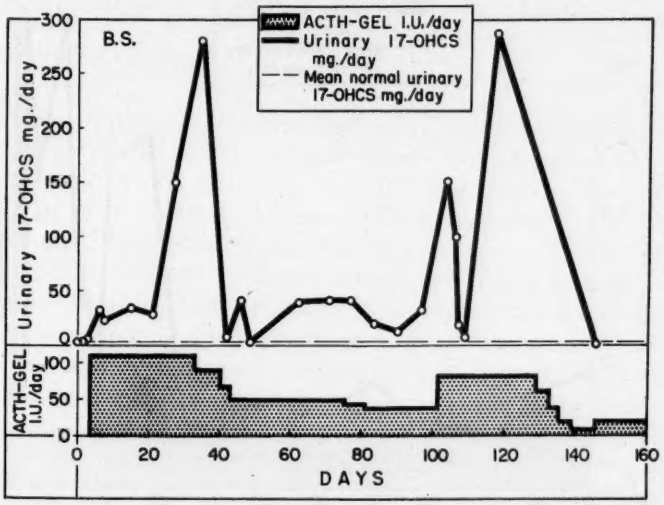

FIG. 2. Variation of urinary 17-OHCS excretion with small change in dose of ACTH-gel.

and of cortisone on urinary excretion of 17-OHCS. In this figure the mean daily urinary 17-OHCS excretion observed in each dose category of each of these drugs (Table 3) is plotted against the size of the dose. For each drug the magnitude of the urinary 17-OHCS excretion was roughly proportional to the magnitude of the dose, permitting the expression of the data by a straight-line curve of reasonably accurate fit. The slope of the straight line representing the relationship between dose of ACTH-gel and 17-OHCS excretion is 1.8, whereas that of the straight line representing the relationship between dose of cortisone and 17-OHCS excretion is 0.5. The ratio of these two slopes is 3.6, indicating that each I.U. of ACTH-gel was equivalent to 3.6 mg. of cortisone in terms of the induced change in urinary 17-OHCS excretion.

The *range* of 17-OHCS excretions during therapy with cortisone and

ACTH-gel at different dose levels is shown in Table 3. Extreme variations occurred at all dosage levels of each drug. Although the magnitude of these variations may be explained partially by the variability in responses among different individuals, there were in many instances variations of similar magnitude among the urinary 17-OHCS excretions of an individual patient on different days while receiving the same daily dose of hormone. This latter point is illustrated by Figures 2 and 3.

In Figure 2 the 24-hour urinary 17-OHCS excretion of Patient B.S. is

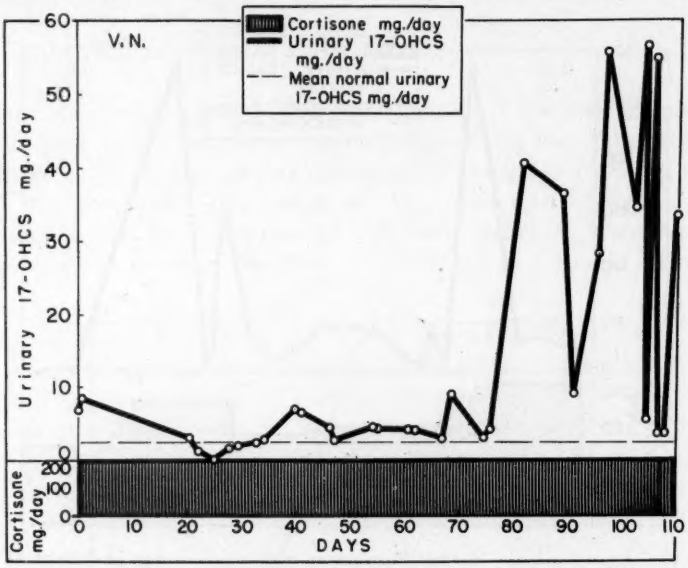

FIG. 3. Variation of urinary 17-OHCS excretion with no change in dose of cortisone.

plotted in relation to the daily dose of ACTH-gel being administered. This graph shows extreme variations in 17-OHCS excretion at times when there was no change or little change in hormone dose. An even more striking illustration of the variations in 17-OHCS excretion which may occur without corresponding changes in hormone dosage is shown in Figure 3. This graph presents the data of Patient V.N., who throughout the study period received a constant dose of 200 mg. of cortisone per day. For seventy-five days this patient excreted very small amounts of 17-OHCS in the urine, despite this large daily dose of cortisone. During the next thirty days, however, rather extreme variations in urinary 17-OHCS excretion occurred and the mean excretion value during this period was much greater than that during the preceding seventy-five days.

TABLE 4. EFFECTS OF SALICYLATE THERAPY ON URINARY 17-OHCS EXCRETION IN PATIENTS WITH RHEUMATIC FEVER

	During therapy	Before therapy	After therapy
No. determinations	23	8	10
No. patients	4	4	3
17-OHCS excretion			
Mean (mg./day)	1.8*	3.1	3.3
SEM	±0.17	±0.35	±0.71
p (*vs.* during therapy)	—	< .01	.05

* Range: 0.1–3.2 mg./day.

In contrast to patients receiving hormone therapy, those receiving salicylate therapy (Table 4) did not excrete increased amounts of 17-OHCS. The mean value in this group was 1.8±0.17 mg. per twenty-four hours, with a range of 0.1–3.2 mg. Apparently these patients had a decreased urinary 17-OHCS excretion during the therapy period. This is confirmed by the data (Table 4) which compare the urinary 17-OHCS excretions during administration of salicylates with those during the pre-therapy and post-therapy periods in 3 rheumatic patients. These data indicate a statistically significant reduction in urinary 17-OHCS excretion during salicylate therapy as compared with the values before and after treatment.

The question of the influence of hormone therapy on subsequent adrenocortical function is of considerable theoretical interest and practical importance. For this reason, a comparison was made of the pre-therapy and post-therapy 17-OHCS excretions in patients receiving different types of treatment for rheumatic fever. These data are presented in Table 5. The pre-therapy urine samples were obtained during the first few days after hospitalization; the post-therapy samples were obtained from two to seven days after discontinuation of the drug. The data in the column headed "All patients" include the results of all determinations made in the pre-

TABLE 5. COMPARISON OF PRE-THERAPY AND POST-THERAPY URINARY 17-OHCS EXCRETIONS IN PATIENTS WITH RHEUMATIC FEVER

	All patients		Cortisone		ACTH-gel		Salicylate	
Therapy status	Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
No. determinations	92	32	15	13	11	6	5	10
No. subjects	30	13	6	6	3	3	3	3
17-OHCS excretion								
Mean (mg./day)	2.0	1.9	2.4	0.68	2.3	2.2	3.0	3.3
SEM	±0.12	±0.34	±0.35	±0.21	±0.94	±0.87	±0.52	±0.71
p(pre- *vs.* post-)	> .5		≪ .01		> .5		> .5	

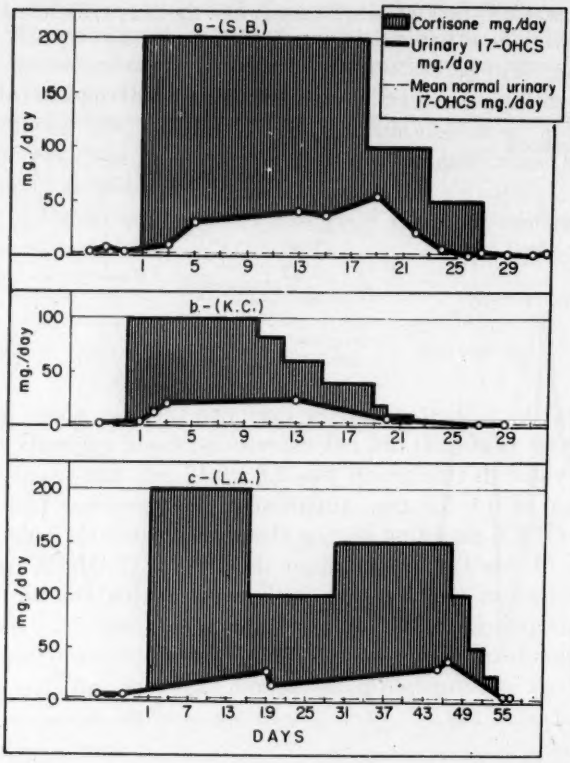

FIG. 4. General urinary 17-OHCS excretion pattern in patients receiving cortisone.

and post-treatment periods, whereas the only individuals whose data are included in the other columns are those for whom both pre- and post-treatment values were obtained. The excretions of 17-OHCS were essentially the same during the post-therapy as during the pre-therapy period in all groups except the one treated with cortisone. Moreover, in all but the cortisone group these values did not differ from that observed in the control group of children. In contrast to the values observed in the other groups in the post-therapy period, the mean value in the cortisone group during this period (0.68 ± 0.21 mg. per day) was significantly lower than that observed in the same group during the pre-therapy period (2.4 ± 0.35 mg. per day), and than that observed in the control group of children (2.2 ± 0.27).

In spite of some extreme variations, as illustrated in Figure 3, most patients receiving each type of therapy exhibited a rather typical urinary 17-

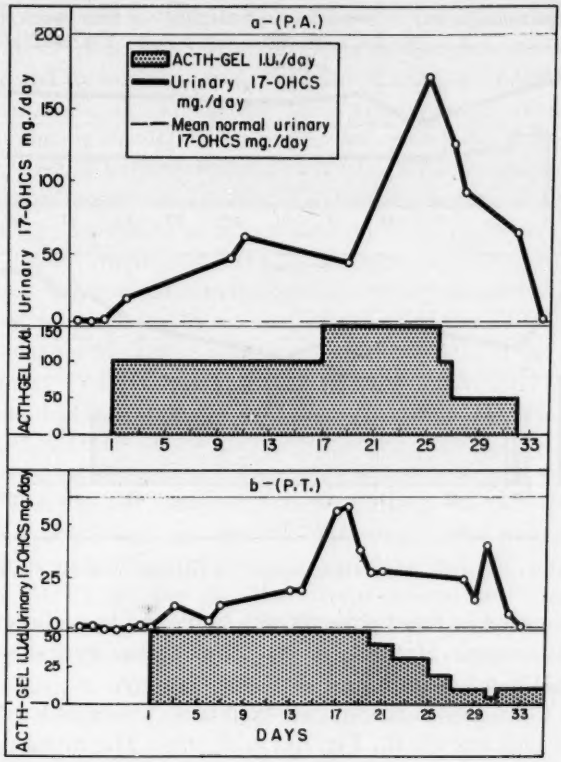

FIG. 5. General urinary 17-OHCS excretion pattern in patients receiving ACTH-gel.

OHCS response pattern. These more typical excretion patterns are presented in Figures 4–6 for illustrative patients receiving different types of therapy. Figure 4 shows the excretion patterns in 3 patients (S.B., Fig. 4a; K.C., 4b; L.A., 4c) treated with cortisone. During the period of therapy these patients all showed elevations in steroid excretions, roughly proportional to the amount of cortisone given. However, the amount of 17-OHCS excreted on any day represented at most only a small proportion of the total amount of cortisone administered that day. The urinary excretion seemed to increase progressively for a few days while the patient was taking the same daily dose of cortisone. This is demonstrated particularly well in Figures 4a and 4b. During the final tapering of the dose and after the discontinuation of therapy the 17-OHCS excretions returned to low values.

The excretion pattern in response of ACTH-gel therapy is shown for 2 representative patients in Figure 5 (P.A., Fig. 5a; P.T., 5b). The pattern

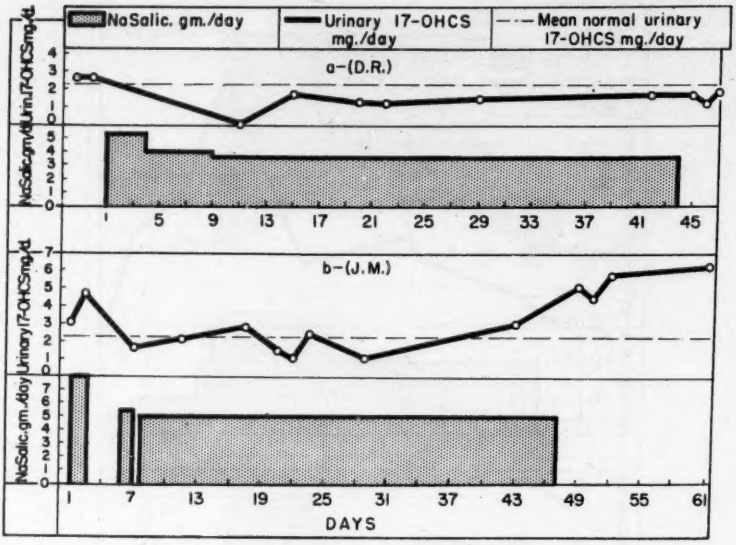

FIG. 6. Urinary 17-OHCS excretion pattern in patients receiving salicylates.

of steroid excretion in these patients was similar to that observed in those treated with cortisone. However the excretion values were somewhat higher during ACTH-gel than during cortisone therapy.

In Figure 6 are shown the steroid excretions associated with salicylate therapy in 2 patients (D.R., Fig. 6a; J.M., 6b). The marked contrast to the steroid excretion patterns observed with cortisone and ACTH-gel therapy is demonstrated by the absence of an elevation in 17-OHCS excretions during the period of salicylate administration. Actually, there was a decrease in these values associated with the administration of salicylates.

DISCUSSION

Previous studies of urinary 17-OHCS excretion have been concerned primarily with adults (1–4, 13–17). In the present study the mean 17-OHCS excretion value observed in young adult males was 5.6 ± 0.63 mg. per twenty-four hours, which is in general agreement with data reported from other laboratories using similar methods. In children 6 to 15 years of age, excretion values were definitely lower (2.2 ± 0.27 mg. per twenty-four hours), despite the fact that the plasma 17-OHCS concentrations in children in this age group do not differ from those in adults (18, 19).

It is accepted generally that the adrenal cortex responds to "stress" stimuli by increasing its secretory activity, although the mechanisms involved are not understood. Severe stress such as that occurring in patients

with acute illness and in terminal conditions causes elevations of plasma 17-OHCS levels (6, 20). In addition, it has been demonstrated that psychic stress is followed by an increase in the rate of urinary 17-OHCS excretion (16). In rheumatic fever, during the "early acute" phase, as in other acute illnesses, the plasma 17-OHCS concentrations are elevated (8). One therefore might expect to find increased urinary 17-OHCS excretion in this disease, since the urinary excretion of 17-OHCS has been proposed as a sensitive index of adrenocortical secretory activity (17). However, the patients with "early acute" rheumatic fever in this series had entirely normal excretion values. If increased adrenocortical activity is present in these patients it is not reflected in the excretion pattern of the 17-OHCS. Proportionality of plasma 17-OHCS response to dose of ACTH (21), and of increase in urinary 17-OHCS excretion to the dose of ACTH, cortisone and hydrocortisone has been reported (22). In patients in this study receiving hormone therapy there was an increased excretion of 17-OHCS. In agreement with previous reports, the average response was proportional to the size of dose of hormone. However, among patients in each dose-group of each hormone, there was considerable variation in the magnitude of excretion. Even among those receiving as much as 300 mg. of cortisone or 70–80 I.U. of ACTH-gel per day there were certain individuals who excreted only small amounts of 17-OHCS on a given day. These variations occurred even in serial determinations on individual patients. Hence, in the individual patient a consistent proportionality does not necessarily exist between the amount of hormone given and the increase in urinary 17-OHCS excretion.

It has been suggested (22) that an increase in adrenal responsiveness is induced by continuous treatment with ACTH, since the same dose of ACTH produces an enhanced urinary excretion of 17-OHCS on successive days if administered over a prolonged period of time. In the present study it was observed similarly that progressive increases in urinary 17-OHCS excretion occurred after several days of continuous ACTH therapy. However, a similar progressive increase in urinary 17-OHCS excretion occurred after several days of continuous cortisone therapy—a form of treatment which could not produce enhanced adrenal responsiveness. These observations of progressive increases in urinary excretion of 17-OHCS during cortisone therapy, combined with the observation that only a relatively small fraction of the administered cortisone appears in the urine, suggest the possibility that mechanisms other than urinary excretion function less efficiently in removing 17-OHCS from the circulation after a period of continuous loading. Further studies will be required to verify this interpretation.

The mean urinary 17-OHCS excretion in the period following cortisone therapy was significantly lower than that in either the same group before

therapy, or the control group of children. In addition, the excretion values observed in the post-therapy period were uniformly lower than those during the pre-therapy period *in each individual* who received cortisone therapy. These findings support the generally accepted belief that cortisone administration inhibits adrenocortical secretory activity. In the ACTH-gel post-therapy group the 17-OHCS excretion values were too few to permit meaningful interpretation.

In patients treated with salicylates there was no apparent difference between pre- and post-therapy urinary 17-OHCS excretions. However, in these patients the mean value for excretion during therapy was significantly lower than either the pre- or post-therapy mean, or the mean observed in the control group of children. This finding is somewhat surprising, since it has been demonstrated (23) that large doses of salicylates in guinea pigs or salicylate intoxication in human subjects causes elevated plasma 17-OHCS concentrations and that these elevations are mediated through pituitary-adrenal stimulation (24). However, it likewise has been demonstrated that salicylates administered to normal human adults in usual therapeutic doses cause an increased rate of removal of 17-OHCS from the blood, which is not attributable to an increased rate of urinary excretion (25). Apparently this latter observation also obtains in patients with rheumatic fever.

SUMMARY

Urinary 17-OHCS excretion has been studied in children with rheumatic fever before, during, and after various types of therapy and compared to that in normal adults and normal children. The values observed in the control group of children were significantly lower than those in the normal adults. In children with rheumatic fever during the pre-therapy period the values observed did not differ from those in the control group of children. The excretion of 17-OHCS was increased during hormone therapy, proportionally to the dose of hormone administered, but with considerable individual variation. Excretion values in the post-cortisone therapy period were depressed significantly. In contrast to the increased urinary 17-OHCS excretions during hormone therapy, the urinary excretions of these steroids during salicylate therapy were significantly lower than those observed during the control periods.

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A METHOD FOR DEMONSTRATING GROWTH HORMONE ACTIVITY IN HUMAN PLASMA*

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WITH an ever increasing interest in the role of pituitary growth hormone (somatotropic factor) in human physiology and more recently in human pathology, it is of utmost importance that a reliable assay method be developed for the demonstration of this hormone in samples of plasma of reasonable size.

This paper is concerned with the fractionation of 1) normal human plasma to which a pure preparation of pituitary growth hormone has been added, 2) human plasma (including that of pregnant women at full term, a patient with acromegaly and a patient with gigantism), and 3) plasma from young pigs and a calf.

The presence of growth hormone in blood has been demonstrated with parabiotic rats by Westman and Jacobsohn (1). They united parabiotically an intact and an hypophysectomized rat. The growth hormone which was carried in the circulation from the intact rat resulted in growth of the hypophysectomized partner.

During the following years several attempts were made to demonstrate growth hormone in human blood. Kinsell and associates (2) injected from 3 to 10 ml. of lyophilized plasma from a patient with gigantism into hypophysectomized female rats and found that the tibial epiphysis was wider than that of either untreated controls or of hypophysectomized rats injected with lyophilized plasma from normal individuals. Greenspan (3) was unable to show any growth hormone activity in pooled lyophilized plasma from 54 growing children.

Since lyophilized plasma did not show any growth hormone activity, Greenspan (3) attempted to concentrate the growth hormone present in plasma. By adding purified growth hormone to plasma and treating it with methanol at 3° C., the hormone was recovered in one fraction. However, by trying the same fractionation on plasma from growing children no growth hormone activity was found. Cotes and Young (4) precipitated

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human plasma to which growth hormone had been added with 50 per cent ammonium sulphate and the hormone was recovered in the lyophilized supernatant liquid. When applying the same technique to normal human plasma, however, no growth hormone was detected.

When using the assay method for growth hormone described by Evans and co-workers (5), the amount of protein injected into each rat must be small in order to avoid toxicity. It is, therefore, desirable to obtain the growth hormone from the plasma in as small an amount of inactive protein as possible. The foregoing method for plasma fractionation will only separate fractions which are too large or too toxic for intraperitoneal injection into small animals.

At present one of the best methods available for plasma fractionation is that of Cohn and associates (6). The separation of the plasma proteins is achieved by precise control of pH, temperature, salt concentration and concentration of protein and organic precipitant. Using this method, which has a high degree of reproducibility, six different fractions may be obtained.

MATERIALS AND METHODS

A growth hormone preparation isolated from ox pituitary by Dr. C. H. Li according to the method of Li, Evans and Simpson (7) was added to freshly drawn human blood. The plasma was separated from the erythrocytes by centrifugation. Fractionation of the plasma was started as soon as possible after the blood was drawn. Three different fractions were separated and called A, B and C. Fraction A was obtained by treating the plasma at $-5°$ C. with 50 per cent ethanol to a final concentration of 20 per cent and adjusting the pH to 6.8 according to the principle of Cohn *et al.* (6). The precipitate formed, which roughly corresponds to Cohn's fractions I, II and III, consists of about 30 per cent of the total amount of protein in the plasma. By changing the concentration of ethanol to 40 per cent and adjusting the pH to 5.8, a precipitate is formed which is called fraction B and consists of about 15 to 20 per cent of the total plasma protein. Fraction C is precipitated when the pH is adjusted to 4.0 at the same temperature and the same concentration of ethanol.

Blood was 1) collected from normal individuals, from the vaginae of a random group of apparently normal women immediately after delivery (retroplacental blood) and from the umbilical cord; 2) drawn from an elderly woman with acromegaly and a young woman with gigantism; and 3) collected in the slaughterhouse from young pigs and a calf. The age of the animals could not be exactly stated but did not exceed 2 months. The plasma was separated by centrifugation and immediately fractionated or lyophilized. A fraction equivalent to 20–40 ml. of plasma or 4–7 ml. of lyophilized plasma was injected into each assay rat.

The tibial epiphyseal cartilage-width test, as proposed by Evans *et al.* (5) and analyzed by Greenspan *et al.* (8), was used as an assay for growth hormone content. Immature female rats, weighing about 60 grams, were hypophysectomized. After a postoperative interval of three weeks the animals received daily intraperitoneal injections of the test sample for four days. Twenty-four hours after the last injection the animals were sacrificed and the two tibiae were dissected free from soft tissue and split with a sharp razor in the midsagittal plane. The bone halves were washed in water and acetone and

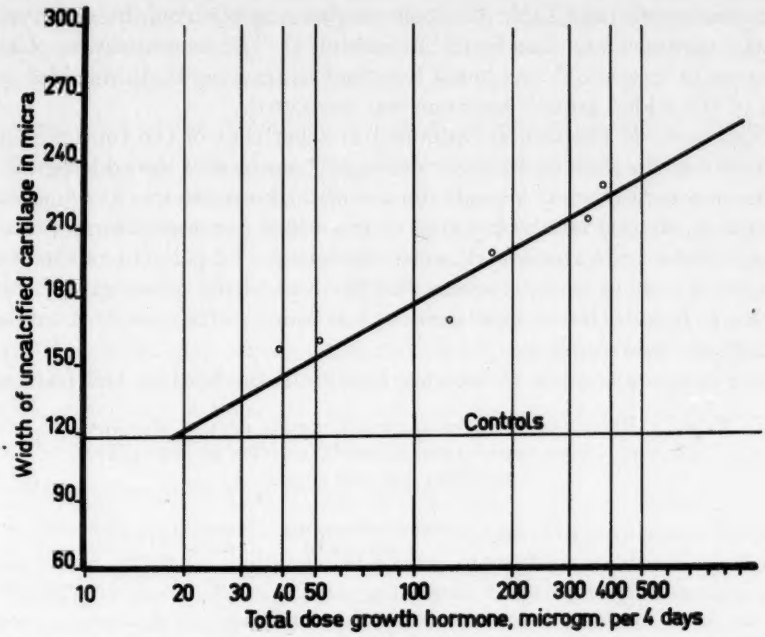

FIG. 1. Effect of pituitary growth hormone on width of the proximal epiphyseal cartilage of the tibia of the hypophysectomized female rat.

stained with a freshly prepared solution of 2 per cent silver nitrate for one and a half minutes. While under water the bones were exposed to a strong light until the calcified portions turned dark brown. The width of the uncalcified epiphyseal cartilage was measured under a microscope, using a calibrated micrometer eyepiece. A minimum of 8 readings were taken on each tibial epiphysis and the results averaged.

A standard assay curve was made, using Dr. C. H. Li's purified growth hormone preparation. In groups of 5 animals given this preparation in doses ranging from 30 to 300 micrograms, the epiphyseal cartilage-width readings were in a straight line when plotted on semi-log paper. The assay method had a lambda of 0.312. A group of 4–5 hypophysectomized animals was used in each assay as controls (Fig. 1).

RESULTS

The growth hormone was recovered in fraction B, which corresponds to Cohn's fractions IV-1 and IV-4. Generally no growth hormone was found in fractions A and C but sometimes the desired percentile limits were difficult to obtain and there was some overlapping.

Recovery of added growth hormone from human plasma

Experiment 1: In fraction B, which consisted of 13.5 per cent of the total amount of protein in the plasma, 17.8 per cent of the added growth hor-

mone was recovered (Table 1). Some overlapping occurred, however, and growth hormone was also found in fraction C. The concentration of the hormone in fraction C was much less than in fraction B. In all, 54.2 per cent of the added growth hormone was recovered.

Experiment 2: Fraction B contained 17.3 per cent of the total amount of protein in the plasma. In this fraction 23.7 per cent of the added growth hormone was recovered. A small amount of the hormone was also found in fraction A. A total of 31.7 per cent of the added hormone was recovered.

Experiment 3: In fraction B, which contained 17.2 per cent of the total amount of protein in the plasma, 28.0 per cent of the added growth hormone was found. Only a small amount was found in fraction A. A total of 37 per cent was recovered.

An attempt was made to separate fraction B further into two fractions

TABLE 1. RECOVERY OF ADDED GROWTH HORMONE FROM HUMAN PLASMA AND THE CONTENT OF GROWTH HORMONE ACTIVITY IN THE PLASMA OF YOUNG PIGS AND A CALF

Expt.	Protein fractions			Amt. protein /assay rat (mg.)	Tibial cartilage width in assay rats (μ)		Growth hormone /assay rat (μg.)	Tot. amt. growth hormone (μg.)	Growth hormone /mg. protein (μg.)	Recovery (%)
	No.	Wt. (Gm.)	%		Range	Avge.				
1. 150 ml. human plasma + 6.65 mg. growth hormone	A	2.98	32.8	27	153, 121, 114, 105	123	0	0	0	0
	IV-1 B	0.39	4.3	30	221, 174, 145, 135	170	90	1,170	3	17.8
	IV-4	0.83	9.2	43	136, 128, 115, 112	123	0	0	0	0
	C	4.85	53.7	90	193, 166, 152, 124, 121	152	50	2,425	0.5	36.4
				Controls:	*135, 132, 130, 120, 112*	*126*				
2. 150 ml. human plasma + 9.50 mg. growth hormone	A	2.55	30.0	172	165, 156, 128	150	50	742	0.3	7.9
	IV-1 B	0.42	5.6	60	211, 160, 141, 133	162	60	300	1.0	3.2
	IV-4	0.97	11.7	96	271, 230, 202, 141, 133	197	190	1,930	1.98	20.5
	C	5.01	52.7	126	158, 123, 100	128	0	0	0	0
				Controls:	*135, 132, 121, 121, 115*	*125*				
3. 15 ml. human plasma + 4.0 mg. growth hormone	A	0.67	40.6	112	183, 162, 145, 138	157	60	359	0.5	9.0
	IV-1 B	0.17	10.0	33	213, 202, 188, 158	191	160	809	4.9	20.5
	IV-4	0.12	7.2	23.5	200, 156, 141, 130	157	60	301	2.5	7.5
	C	0.70	42.2							
				Controls:	*125, 102, 93*	*106*				
4. 210 ml. plasma from young pigs	A	5.31	36.2	500	115, 112	114	0	0	0	0
	B	0.95	6.5	240	153, 141, 125, 116	134	30	120	1.25	57 μg. /100 ml. plasma
	C	8.31	57.3							
				Controls:	*120, 120, 115, 111, 102*	*114*				
5. 995 ml. plasma from a calf	A	26.0	39.1	500	140, 130, 120	130	0	0	0	0
	B	4.33	6.5	500	161, 142, 140, 140	146	42	363	0.84	37 μg. /100 ml. plasma
	C	36.1	54.4							
				Controls:	*135, 127, 118, 117*	*125*				

which, according to Cohn, may be called IV-1 and IV-4. The growth hormone activity, however, was found alternately in the two fractions and a separation of this sort was not suitable.

Determination of growth hormone activity in plasma of young pigs and a calf

Pooled plasma from young pigs was fractionated as described. In fraction B, which consisted of 6.5 per cent of the total amount of protein in the plasma, growth hormone activity was found. The effect on the assay rats corresponded to that which would be expected from 120 μg. of the purified growth hormone preparation. A large amount of plasma from a calf was also fractionated. In fraction B, 6.5 per cent of the total amount of protein was precipitated and, when tested, gave a response equivalent to that induced by 363 μg. of the purified preparation. The concentration of growth hormone activity in these two plasma samples was 57 and 37 μg. per 100 ml. of plasma, respectively (Table 1).

Growth hormone activity in normal human blood

Several attempts were made to demonstrate growth hormone activity in the plasma of normal individuals. A large amount of fractionated or lyophilized plasma was injected into each assay rat without producing an increase in the width of the tibial epiphyseal cartilage (Table 2).

Growth hormone activity in retroplacental plasma

Fractionated plasma from retroplacental blood, in amounts equivalent to 18.5 to 43.0 ml. of untreated plasma, when injected into each assay rat gave an increase in the width of the proximal tibial epiphysis which, when compared to the standard dose-response curve, corresponded to the effect of 40–130 μg. By simply injecting lyophilized plasma from retroplacental blood, a positive response was obtained with doses equivalent to 7 to 15 ml. of untreated plasma. The concentration of growth hormone activity in

TABLE 2. CONTENT OF GROWTH HORMONE ACTIVITY IN NORMAL HUMAN PLASMA

Treatment of plasma	Amt. plasma /assay rat (ml.)	Amt. protein assay rat (mg.)	No. assay rats	Tibial cartilage width in assay rats (μ)		Growth hormone /assay rat (μg.)	Growth hormone /mg. protein (μg.)	Growth hormone /100 ml. plasma (μg.)
				Range	Avge.			
Fractionated (fract. B)	23.5	300	3	126, 120, 96	114	0	0	0
			Controls	*127, 123, 105, 94*	*112*			
Lyophilized	4.3	300	4	142, 140, 139, 124	136	0	0	0
			Controls	*142, 141, 139, 136*	*139*			
Lyophilized	7.0	500	4	150, 140, 135, 121	136	0	0	0
			Controls	*141, 136, 130, 126, 124*	*139*			
Lyophilized	7.0	500	3	145, 136, 133	138	0	0	0
			Controls	*145, 131, 110*	*129*			

TABLE 3. CONTENT OF GROWTH HORMONE ACTIVITY IN PLASMA FROM THE VAGINAE OF WOMEN IMMEDIATELY AFTER DELIVERY (RETROPLACENTAL BLOOD)

Treatment of plasma	Amt. plasma /assay rat (ml.)	Amt. protein /assay rat (mg.)	No. assay rats	Tibial cartilage width in assay rats (μ)		Growth hormone /assay rat (μg.)	Growth hormone /mg. protein	Growth hormone /100 ml. plasma (μg.)
				Range	Avge.			
Fractionated (B)	21.5	150	4	144, 132, 120, 107	126	0	0	0
			Controls	*138, 127, 115, 112*	*123*			
Fractionated (B)	35.0	250	2	180, 138	159	65	0.26	185
			Controls	*126, 126, 114, 115*	*120*			
Fractionated (B)	43.0	300	4	244, 186, 158, 150	185	130	0.43	300
			Controls	*123, 116*	*120*			
Fractionated (B)	18.5	500	2	156, 132	144	40	0.08	220
			Controls	*123, 116*	*120*			
Lyphophilized	4.3	300	3	126, 120, 96	114	0	0	0
			Controls	*127, 123, 105, 93*	*112*			
Lyophilized	7.0	500	3	185, 167, 126	159	65	0.13	925
			Controls	*109, 109*	*109*			
Lyophilized	7.0	500	4	171, 142, 132, 126	144	40	0.08	570
			Controls	*123, 116*	*120*			
Lyophilized	11.0	780	3	168, 166, 166	167	78	0.10	710
			Controls	*147, 131, 108*	*129*			
Lyophilized	14.0	1,000	3	150, 138, 135	141	39	0.04	280
			Controls	*124, 120, 112*	*118*			
Lyophilized	15.0	1,100	4	175, 166, 156, 147	162	70	0.06	450
			Controls	*135, 130, 124, 117, 117*	*124*			
Lyophilized	7.0	500	4	175, 166, 161, 145	162	70	0.14	1,000*
			Controls	*140, 149, 144, 136, 125*	*138*			

* Diabetic mother.

retroplacental blood was 185–300 μg. per 100 ml. of plasma after fractionation, and 280–925 μg. per 100 ml. after simply lyophilizing the plasma. Lyophilized plasma of retroplacental blood from a woman with diabetes mellitus showed a concentration of growth hormone activity equivalent to 1,000 μg. per 100 ml. of plasma (Table 3).

Growth hormone activity of blood from the umbilical cord

With lyophilized plasma in doses equivalent to 4.6 to 7.0 ml. of plasma in all cases there was a positive response to the tibia test. The amount of growth hormone activity was equivalent to 38 to 48 μg. of the standard preparation. The average concentration of growth hormone activity in lyophilized plasma from the umbilical cord was 650 μg. per 100 ml. of plasma. This was about the same concentration found in retroplacental blood collected at delivery (590 μg. per 100 ml. of plasma) (Table 4).

Growth hormone activity in the plasma of a patient with acromegaly and a patient with gigantism

With lyophilized plasma from these 2 patients, injected in doses equivalent to 5.7 to 7.0 ml. of plasma, there was a positive response to the tibia

TABLE 4. CONTENT OF GROWTH HORMONE ACTIVITY IN PLASMA FROM THE UMBILICAL CORD

Treatment of plasma	Amt. plasma /assay rat (ml.)	Amt. protein /assay rat (mg.)	No. assay rats	Tibial cartilage width in assay rats (μ)		Growth hormone /assay rat (μg.)	Growth hormone /mg. protein (μg.)	Growth hormone /100 ml. plasma (μg.)
				Range	Ave.			
Lyophilized	4.6	320	4	165, 143, 135, 120	141	39	0.12	850
			Controls	*112, 100*	*106*			
Lyophilized	7.0	500	4	153, 150, 130, 128	141	39	0.08	560
			Controls	*124, 120, 112*	*118*			
Lyophilized	7.0	500	3	147, 142, 127	140	38	0.08	540
			Controls	*129, 126, 124*	*126*			
Lyophilized	7.0	500	3	148, 148, 145	147	45	0.09	640
			Controls	*129, 126, 124*	*126*			

test. Two assays performed on the plasma from the patient with gigantism gave similar results (Table 5).

DISCUSSION

The results of the attempts to extract added growth hormone from plasma seem to show that about 25 per cent of the added growth hormone is found in 15 per cent of the total plasma protein. Hence a large portion of the added growth hormone can not be recovered. It may be destroyed during the fractionation or may be lost in the other two fractions. As the maximum dose of fractionated plasma protein which may be given to each assay rat is about 500 mg., and the smallest dose of growth hormone which will induce a positive response is 30 μg., it is necessary to have 120 μg. of growth hormone in 50 ml. of plasma to get a positive response. Thus, the lowest concentration of growth hormone which may be detected in plasma by the tibia test is 240 μg. per 100 ml. of plasma.

By simply lyophilizing plasma, a growth hormone concentration of 500 μg. per 100 ml. of plasma is needed in order for a dose of 500 mg. to induce a positive response to the tibia test. Since the assay rats seem to tolerate lyophilized plasma better than fractionated plasma, larger doses may be

TABLE 5. CONTENT OF GROWTH HORMONE ACTIVITY IN THE PLASMA OF A PATIENT WITH GIGANTISM AND A PATIENT WITH ACROMEGALY

Treatment of plasma	Amt. plasma /assay rat (ml.)	Amt. protein /assay rat (mg.)	No. assay rats	Tibial cartilage width in assay rats (μ)		Growth hormone /assay rat (μg.)	Growth hormone /mg. protein (μg.)	Growth hormone /100 ml. plasma (μg.)
				Range	Avge.			
Lyophilized (gigantism)	7.0	500	4	158, 157, 151, 150	155	54	0.11	770
			Controls	*140, 139, 138, 126*	*135*			
Lyophilized (gigantism)	7.0	500	4	166, 160, 145, 140	153	53	0.11	760
			Controls	*140, 126, 120*	*128*			
Lyophilized (acromegaly)	5.7	400	4	166, 162, 162, 159	162	70	0.17	1,190
			Controls	*145, 145, 127*	*139*			

administered. However, these calculations are made with the assumption that no growth hormone activity is destroyed by the lyophilization procedure.

If untreated plasma is injected into the assay rats a maximum dose of 4 ml. may be given during the four-day period. Under these conditions the concentration of growth hormone in the untreated plasma must be about 800 μg. per 100 ml. of plasma in order to induce a positive response to the tibia test.

The results of Kinsell *et al.* (2) have been confirmed in this study. Lyophilized plasma of a patient with acromegaly and from a young woman with gigantism (23 years of age) showed growth hormone activity, whereas no activity could be found in plasma of normal individuals. The blood levels of growth hormone activity reported by Kinsell *et al.* were much lower than those found in this study. This may be partly due to a difference in the activity of the reference standards used in the two studies. The minimum effective dose of growth hormone standard used by Kinsell *et al.* was 5 μg., whereas the minimum effective dose of the growth hormone preparation used in this study was 30–40 μg.

Since the retroplacental plasma and the plasma of the umbilical cord blood have almost the same amount of growth hormone activity, it may be suggested that the placenta is permeable to growth hormone. The high concentration of this activity may suggest that it is produced in the placenta, although the mother's pituitary, which increases in size during pregnancy, may also be the production site. An attempt was made to concentrate growth hormone activity from the placenta. The placenta was immediately frozen and prepared according to a method described by Wilhelmi, Fishman and Russell (9) for isolation of growth hormone from pituitaries. Several fractionations of this kind were performed, but only on two occasions could growth hormone activity be demonstrated.

Since the tibia test is not specific for growth hormone, it is not possible from these results to draw any conclusion about the identity of the substance concentrated from human plasma or from the plasma of young pigs and a calf. Thyroxine (10), thyrotropic hormone (11) or insulin (12) will enhance the effect on the tibia epiphysis, whereas cortisone or ACTH (13) will inhibit the effect. One or several of these substances may be present in the plasma and be concentrated along with the growth hormone. Cohn (14) has shown that thyrotropic hormone precipitates in fraction IV-4. For identification of the growth-promoting factor concentrated from the plasma, further experiments are necessary. Unfortunately the amount of this substance in plasma seems to be so small that assay at different dose levels may be difficult to accomplish.

It has been shown by Gemzell (15) that large amounts of 17-hydroxycor-

ticosteroids are present in the retroplacental blood and in the blood from the umbilical cord. As these steroids are stable and are not destroyed by the lyophilizing technique, they probably inhibit the effect of growth hormone on the tibial epiphyseal cartilage. The results obtained with the lyophilized plasma of retroplacental blood may therefore be too low. Both fractionated and lyophilized plasma of the retroplacental blood and umbilical-cord blood have been assayed for ACTH with the adrenal ascorbic-acid depletion method of Sayers, Sayers and Woodbury (16) without any ACTH activity being demonstrated. Other hormones, such as estrogens and gonadotropins which are present in large amount in pregnancy, have no effect on the width of the cartilage.

SUMMARY

A method has been described for concentration of growth hormone added to human plasma. The plasma was fractionated according to the method of Cohn *et al.* (6). The added growth hormone was recovered in fraction B (Cohn's fractions IV-1 and IV-4). About 25 per cent of the hormone was recovered in 15 per cent of the total plasma protein.

In fractionated plasma of young pigs and a calf, growth hormone-like activity was demonstrated in a concentration of 40–50 μg. per 100 ml. of plasma.

No growth hormone activity was found in fractionated, or lyophilized plasma from normal human blood.

Growth hormone-like activity was demonstrated in fractionated or lyophilized plasma of retroplacental blood, in plasma from the umbilical cord blood and in plasma from 2 patients with acromegaly and gigantism.

At present it is impossible to say whether this growth-promoting activity is identical with that of the pituitary growth hormone.

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HEREDITARY DIABETES INSIPIDUS:

REPORT OF 20 CASES IN SEVEN GENERATIONS*

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INTRODUCTION

THE familial occurrence of diabetes insipidus was first reported over one hundred years ago by La Combe in 1841, as cited by Forssman (1). At that time Mendel's laws of inheritance had not yet been described. Since then, about 35 reports with case analyses and family trees have appeared in the literature, primarily from Europe. The studies carried out by the Weils, Sr. and Jr., and summarized by Camerer (2) in 1935, are a classic reference on the mode of transmission of this anomaly, stressing the presence of a dominant gene. More recent comprehensive surveys are those of Forssman in Sweden in 1945 (1) and Pender and Fraser in Canada in 1953 (3). There have been only a few reports from this country (4–6), and it is the purpose of this paper to present a family history in which individuals of seven generations were affected by the disease and to discuss the genetic aspects of inherited diabetes insipidus.

CASE REPORT

Our interest in this subject was aroused by encountering a patient (*V*:1 in the pedigree, Fig. 1) who had diabetes insipidus and who had a remarkable history of frequent occurrence of the disease in her family. By personal interview with a few members of her family, but principally by extensive correspondence with many, it was possible to gather sufficient data (usually cross-checked from several sources) to build up a family pedigree (Fig. 1). The case history of the patient follows in some detail; short summaries of the histories of several other members of the family are given in Table 1.

Mrs. A. A. (UCH 20242), V: 1 in the pedigree, was first seen in the Endocrine Clinic of the University of California Hospital by one of us (R. E.) in October 1937. She was then 26 years of age and complained of unusual thirst and urinary frequency since her

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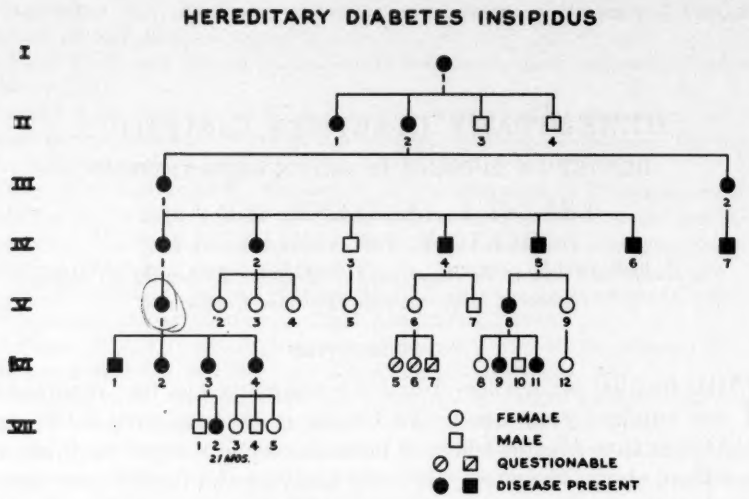

FIG. 1. Pedigree, showing distribution of cases. Note absence of skipped generations, denoting transmission by simple dominance.

earliest memory. Her urinary output at that time measured 13¼ quarts in twenty-four hours. In discussing her family history the patient stated that the "water habit" was a family characteristic which had been manifested by her mother, grandmother, great-grandmother, and great-great-grandmother, as well as by her maternal aunts and 3 of her 4 uncles. Her 3 children also were affected. She recalled that at family gatherings during her childhood, she and the other children lined up at bedtime to receive the water they desired before retiring. At the age of 7 years, the patient had an attack of rheumatic fever.

TABLE 1. INFORMATION ON INDIVIDUAL CASES IN FAMILY

I:1	First generation, No. 1. Great-great-grandmother of patient, reported to have suffered from excessive thirst and urinary frequency. Was in the habit of going to the creek in middle of the night to get bucket of water, and was known to drink a half bucketful at a time.
II:2	Great-grandmother of patient. Was in the habit of visiting the well during the night to replenish her supply of drinking water.
III:1	Grandmother of patient. Reported to have had great thirst and to have been "greedy and hoggish about water." Several brothers and sisters were said to have had the disease, but sufficient data were not available to warrant their inclusion here.
III:2	Female. Said to have had the disease severely; suffered from great thirst and urinary frequency. Died of stroke.
IV:1	Patient's mother. Died at age 19 of an accident; no data available, other than the fact that she had the disease.

IV:2 Female. Excessive thirst and urinary frequency since her earliest memory. Water intake up to 24 quarts daily. Controlled by treatment for a time but now "accustomed to it." General health good at age 53.

IV:4 Onset in childhood, with great thirst and urinary frequency. No medical treatment. This man had a favorite dipper, still kept by his daughter, and found recently to hold 1½ cups (360 cc.); usually drank one or two dipperfuls at a time. Died at age 53 of intestinal neoplasm. Last words were, "It's a long time between drinks."

IV:5 No detailed information regarding this man. He had diabetes insipidus, and died at the age of 36.

IV:6 Male. Excessive thirst, urinary frequency and polyuria since his earliest memory. Takes up to 16 quarts of fluid per twenty-four hours, some of which is consumed during the night. Greedy about water, and deprivation causes "suffering." Living at age 58, but has arthritis.

IV:7 A man who is reported to be a heavy drinker of water. Now lives in Michigan.

V:1 *Patient whose history is given in this report.*

V:8 Onset of disease in infancy. This woman is now 25 and drinks at least 6 quarts of liquids daily. She had a marked increase of thirst during pregnancy and may have drunk 1 or 2 quarts at a time. Had constant craving to chew ice; at times thirst seemed unquenchable. The pattern was the same with each of four pregnancies. She also has epidermolysis bullosa, as have 3 of her 4 children. There was a good response to Pitressin®.

VI:1 Onset at 5 months of age, with excessive thirst and urinary frequency. This boy is now 14 years old and drinks 10 to 12 quarts of liquid daily. His general health is fair, but he is subject to migraine headaches and asthma, and requires a home teacher.

VI:2 Excessive thirst, urinary frequency and drowsiness appeared at 1 year of age. This woman is now 25 and drinks 13 quarts of fluids daily. Increased thirst noted with recent pregnancy.

VI:3 Onset in infancy. This 22-year-old woman drinks 16 quarts of water daily. Thirst and urinary frequency were increased during each of two pregnancies. There was a dramatic response to Pitressin® while she was at the University of California Hospital recently for delivery.

VI:4 Female. Excessive thirst, frequent urination and drowsiness appeared at the age of 8 months. She takes 10 quarts of liquid daily; prefers ice water and ice-cold beverages. Thirst increased during each of three pregnancies. Children have not shown symptoms so far.

VI:5, 6 & 7 Two girls and 1 boy, aged 8, 10 and 11 years, respectively. They drink more than average amounts of water, but only 1 (VI:7) suffers from nocturia. Questionable cases.

VI:9 A 2-year-old girl. Drinks increasing amounts of water as she grows older. Cries for water several times during the night.

VI:11 A 7-year-old girl. Drinks large amounts of water; gets up several times each night to drink water and urinate.

VII:2 A 21-month-old girl, who needs large amounts of water. Can be soothed with water if crying. Sits on floor and drinks almost continuously if allowed to do so. First word learned was "wa-wa."

On physical examination, the only positive findings were moderate obesity and an apical systolic heart murmur. On laboratory examination, urine specific gravities were 1.001 to 1.003 and sugar and albumin were absent. X-ray films showed a normal skull. Kahn and Wassermann reactions were negative.

Nasal insufflation of posterior pituitary powder, one "pinch" (about 15 mg.) two to four times daily, adequately controlled her symptoms. She had a fourth child in 1939 without difficulty. In 1945, at the age of 36, she experienced the first episode of cardiac decompensation due to rheumatic heart disease. This improved with bed rest and digitalization, but there were recurrences in 1947 and 1953. During the last attack she was hospitalized and, while convalescent, was given a Carter-Robbins test (7) (a modification of that of Hickey and Hare (8)), which definitely demonstrated that her "water habit" was indeed true diabetes insipidus (Fig. 2). Following this episode it was decided to allow the

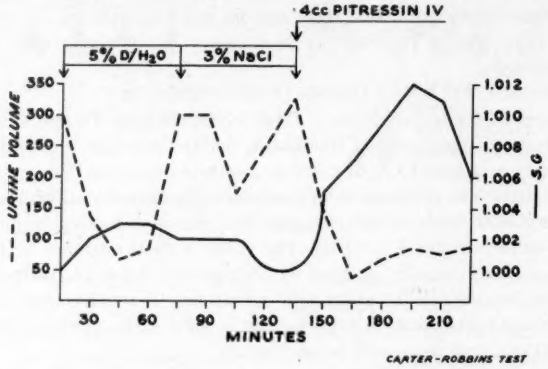

FIG. 2. Carter-Robbins test on Patient A.A. (V:1 in pedigree). Note lack of effect of intravenous hypertonic saline on rate of urine flow (cc. per 15 minutes). When Pitressin® was administered, there were characteristic changes, *i.e.*, decreased rate of urine flow and an increase in specific gravity.

diuresis due to her diabetes insipidus to proceed unimpeded in order to offset the tendency toward fluid retention from congestive failure. Therefore, the posterior pituitary therapy was gradually withdrawn. She got along fairly well on this routine, which also included limitation of activity, restriction of salt, and maintenance dosage of digitalis. However, nocturia (two or three times) was bothersome. A later complication was chronic diarrhea with loose stools and mucus four or five times daily. Various studies did not adequately explain the cause of this symptom, and it was therefore decided to reinstitute treatment with posterior pituitary powder. A dramatic improvement in the diarrhea resulted, and the nocturia ceased. The patient is now comfortably maintained with two or three nasal insufflations of the powder daily, in addition to routine medication for her cardiac condition.

DISCUSSION

The diagnosis of diabetes insipidus appeared certain in this patient from her history, symptomatology and from the specific reaction to Pitressin®

in the Carter-Robbins test. Although most of the data on the disease in the scattered members of our patient's family were obtained by correspondence, they were derived from enough different sources, with agreement on cross-checking, to convince us of their validity for this report. The improvement of the diarrhea during antidiuretic therapy is difficult to explain, since diarrhea is not a usual symptom of diabetes insipidus. One possibility is that it may have been a manifestation of an irritable gastrointestinal tract caused by general stress and poor health.

Inheritance

Concerning the genetic aspects of the disease, study of many of the early pedigrees, beginning with those reported by Weil (9) and summarized by Camerer (2), suggested that the transmission was by simple dominance. Subsequently, other authors commented on the phenomenon of skipped generations and added the qualification of "incomplete penetrance." The most controversial point regarding the mode of transmission of diabetes insipidus arose with the introduction of the theory of sex-linkage. Forssman (1) reported several pedigrees, in fact the largest in his series, in which the condition occurred only in males, the females acting merely as carriers (as in hemophilia). He believed that these females were heterozygotes, but were phenotypically normal. Some of them had "diabetes insipidus gravidarum," *i.e.*, the disease occurred only during pregnancy and then disappeared. (This increase in symptoms during pregnancy was also noted by several women in our series.) Others had very mild diabetes insipidus, and the rest were completely normal. However, several of the women in his series who had no signs or symptoms of the disease did have sons in whom the condition appeared—a strong argument in favor of sex-linkage in this particular pedigree. In another series published by Williams in the United States in 1947 (10), sex-linked inheritance seemed well established. His cases were of interest inasmuch as they did not respond to Pitressin®. This variation of the disease is called "nephrogenic diabetes insipidus."

Opinions expressed in the majority of the reported series and those of writers on medical genetics (11–14) agree that transmission of the disease is by a "conditionally dominant gene with poor penetrance." The family described in this report (Fig. 1 and Table 1) would seem to fall into the general category of transmission by a dominant gene with poor penetrance without evidence of sex-linkage. There has not yet been an instance of a "skipped generation," denoting that the gene is strongly autosomal (simple dominance). There are 20 non-affected and 20 affected persons in our series, representing a ratio of 1:1, as is usually found in simple dominance. The doubtful cases in the last generations are in young children, and

a diagnosis of diabetes insipidus may become definite in later life, since pronounced manifestations are sometimes not seen until the second decade.

The present series represents one of the largest reported in the United States to date, although a report being prepared by Cannon is much more extensive (15).

SUMMARY

A female patient with diabetes insipidus is described. Urinary output was 13½ quarts daily, and the results of the Carter-Robbins test were positive. The course of the disease was complicated by rheumatic heart disease with decompensation and, later, by chronic diarrhea of undetermined origin. The latter improved after reinstitution of antidiuretic therapy. There were many instances of the disease on the maternal side of the family. Investigation by correspondence and interviews elicited a pedigree with 20 cases in seven generations. Genetic analysis showed that the transmission in this family was by simple dominance. This agrees with the opinions expressed in most reports of similar series in the world literature.

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HYPOPHYSECTOMY IN MAN: EFFECT ON WATER EXCRETION DURING THE FIRST TWO POSTOPERATIVE MONTHS*

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THE role of the hypothalamic-hypophyseal axis in water excretion has been extensively studied in animals. Our knowledge on this subject in man is principally derived from observations in pathologic conditions involving this area. The significance of such observations may, however, always be questioned, as the extent of the damage cannot often be defined with accuracy. Hypophysectomy in man (1, 2) provides material in which the influence of the hypophysis on water excretion can be studied under well defined conditions.

The aim of the present paper is to report on the changes in the daily urine volume and the concentrating and diluting ability of the kidney of hypophysectomized patients during the first two months after operation.

MATERIAL AND METHODS

In the selection of hypophysectomized patients for the present study, those subjects were excluded who showed an incomplete hypophysectomy, altered kidney function, extensive liver metastases or large collections of fluid in the serous cavities. The material consisted of 24 patients, 4 males and 20 females, between the ages of 30 and 69 years. Two of the males had metastatic cancer of the prostate; all the other patients had advanced metastatic cancer of the breast. The hypophysectomy was performed as described previously (2). According to this procedure a silver clamp was put in the middle of the hypophyseal stalk, which was then severed at a point distal to the clamp.

The daily urine volumes in all patients were collected from 8 A.M. to 8 A.M. The patients were all in general wards, except for a few who were studied in the metabolic ward. The room temperature varied between 20° and 25° C. An indwelling catheter in the bladder was used during the first postoperative days, and later on whenever necessary. The urines were kept in an ice-box in stoppered bottles, without the addition of preservatives.

The water-load test was performed according to the method of Luft and Sjögren (3). No food or water was allowed after 10 P.M. of the previous day. The night urine was collected until 8 A.M. The patient then received 1000 ml. of water by mouth between 8:00

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and 8:30 A.M. The bladder was emptied at hourly intervals. Patients having polyuria were allowed water without restriction during the previous night, but they were instructed to avoid excessive drinking between 5 and 8 A.M.

The thirst test was performed as follows: The patient emptied the bladder at 8 A.M., and had breakfast between 8 and 8:30 A.M. No fluid was allowed during the following twenty-four hours. The urine was collected in 4-hour portions during the whole test.

For the intravenous water-load test a catheter was inserted in the bladder, and a timed urine specimen was collected between 8:30 and 9:30 A.M. After emptying the bladder, 1 liter of 5.5 per cent glucose was given by intravenous infusion in forty-five minutes. The bladder was emptied every half hour for three hours after the start of the infusion.

Spontaneous changes in the concentration of the urine over a 24-hour period were followed by studying the changes in specific gravity and freezing point depression in the urine collected in 4-hour portions, while the intake of food and fluid of the patients was unrestricted.

The number of patients used for each study is given in the section on results.

The freezing-point depression of the urine was measured by a Beckman thermometer. The 17-ketosteroids and corticoids in urine were measured by the methods of Hamburger (4) and Norymberski *et al.* (5), respectively.

RESULTS

Daily urine volumes

The daily urine volume increased after hypophysectomy from a preoperative level of about 1 liter to a level of between 1.7 and 2.7 liters, with a minimum value around the fifth day and a maximum around the twelfth day. The variations were large, not only in different individuals on the same day but also in the same individual from day to day (Figs. 3 to 5). Values within the preoperative range were also observed for some days in most of the patients. In 2 cases the daily urine volume after operation never exceeded 2 liters. The over-all change in the daily urine volume in each case was a polyuria for the initial few days after the operation (first polyuric phase), followed by a decrease in the diuresis toward or to the preoperative level (oliguric phase), which was then succeeded by a second polyuric phase. These changes are illustrated in Figures 1 and 2, in which the highest values during the first and second polyuric phase as well as the lowest values during the oliguric phase are plotted. The mean for each group of values was calculated. The line connecting the plotted mean values is arbitrarily drawn. The first peak in the diuresis was found on the third day (second to sixth), amounting to 2.8 liters (1.2 to 5.0 liters); the lowest volume on the seventh day (third to eleventh), amounting to 1.1 liters (0.5 to 2.9 liters); and the peak during the second diuretic phase on the fifteenth day (seventh to twenty-first), amounting to 3.9 liters (1.4 to 9.0 liters).

As shown in Figure 2, the patients were classified according to treatment, as follows: Group 1—no substitution; Group 2—cortisone for the first five

URINE VOLUME
LITERS PER 24 HOURS

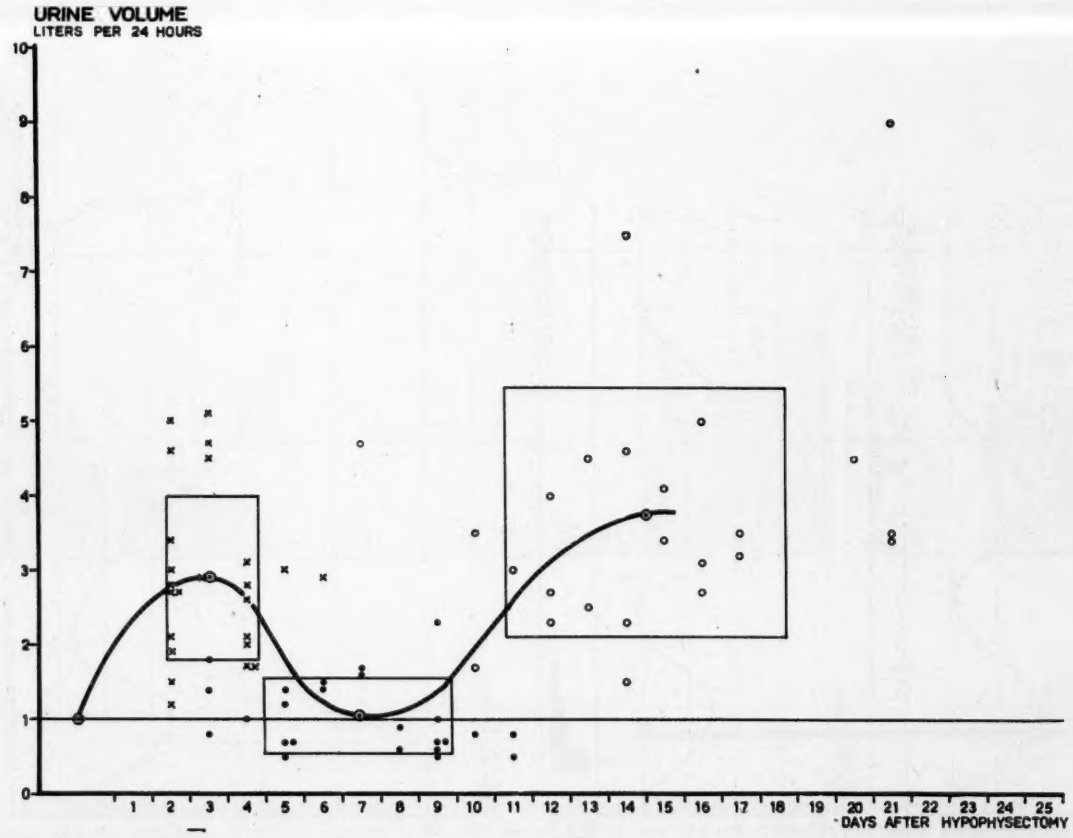

FIG. 1. Changes in diuresis after hypophysectomy in man. × denotes the largest daily urine volume during the first week; ● denotes the lowest daily urine volume during the oliguric phase; ○ denotes the largest daily urine volume observed after the oliguric phase; ⊙ denotes the mean of each group of values.

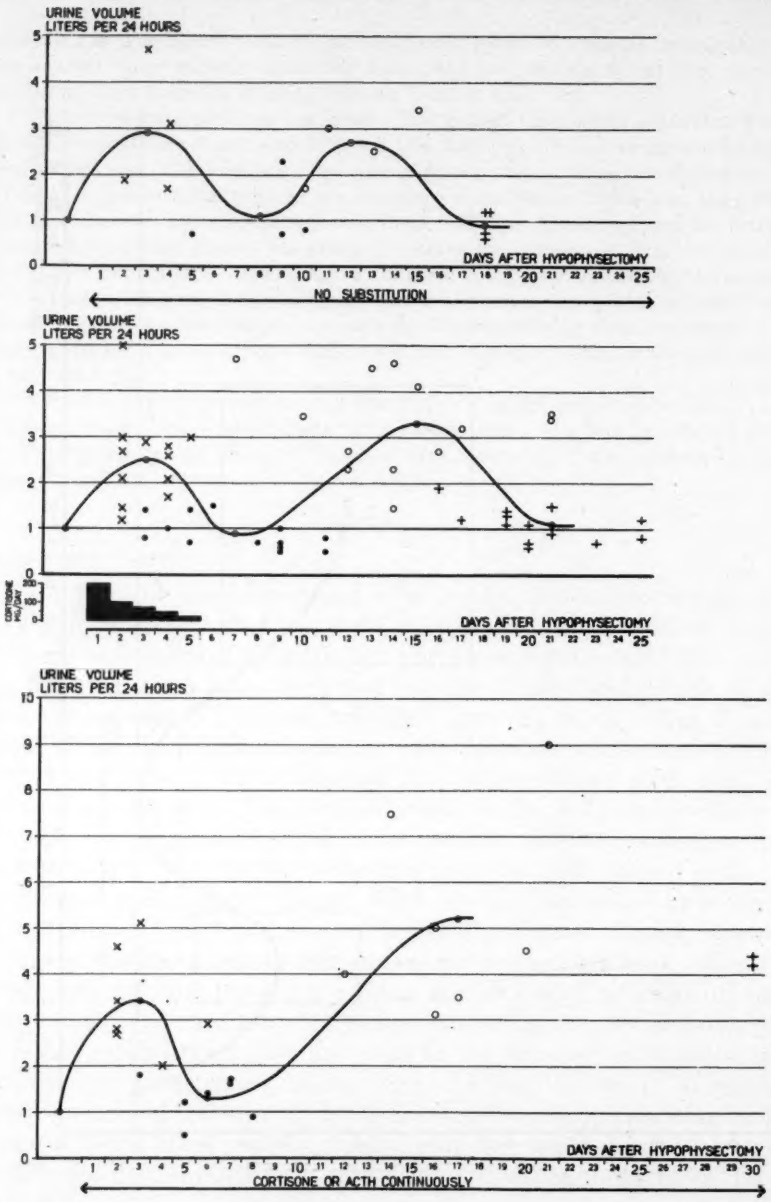

FIG. 2. The influence of the postoperative administration of cortisone or ACTH on changes in diuresis after hypophysectomy in man. ×, ●, ○, and ⊙ as in Figure 1. + denotes the lowest daily urine volume after the second diuretic peak. *Top:* Group 1, no substitution. *Center:* Group 2, cortisone for the first five postoperative days. *Bottom:* Group 3, cortisone or ACTH continuously.

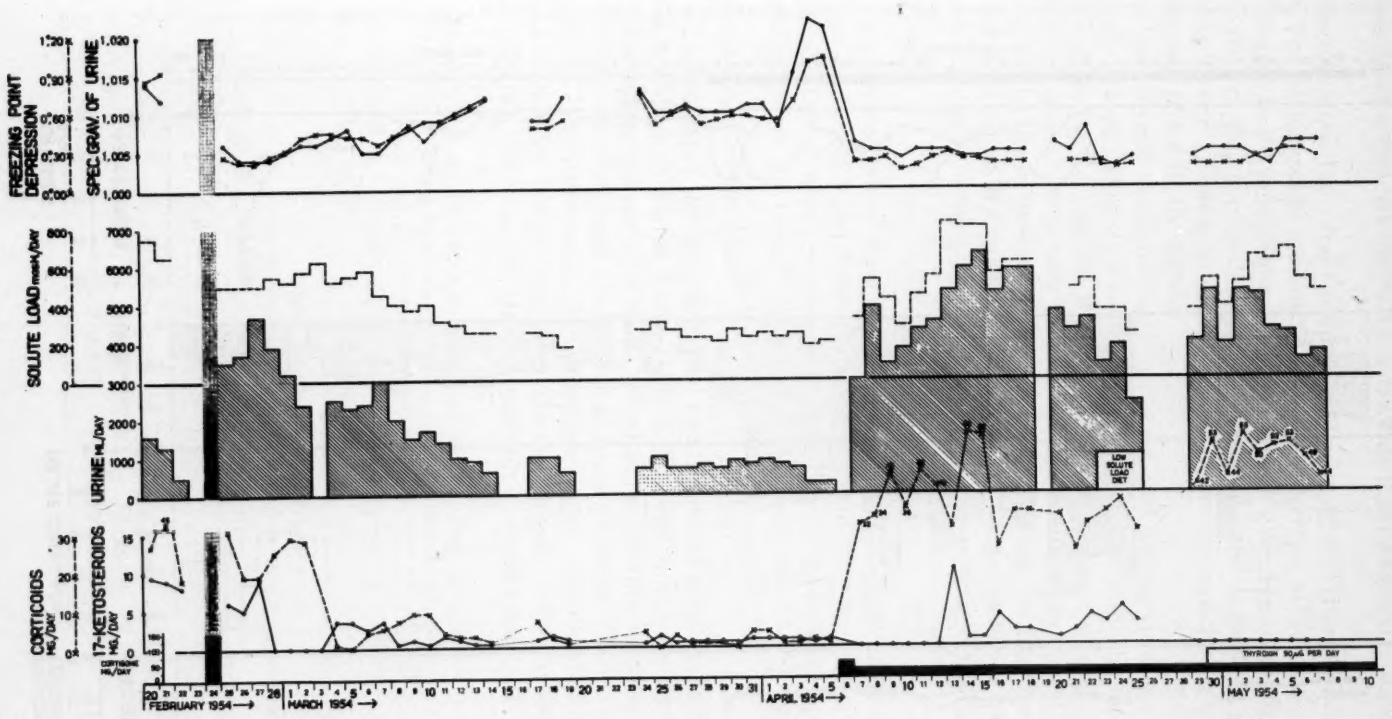

FIG. 3. Daily urine volume, effective solute load, excretion of 17-ketosteroids and corticoids, as well as changes in the specific gravity and osmolarity of the urine after hypophysectomy in a woman of 34 with metastatic cancer of the breast. No cortisone was given for forty days after the operation.

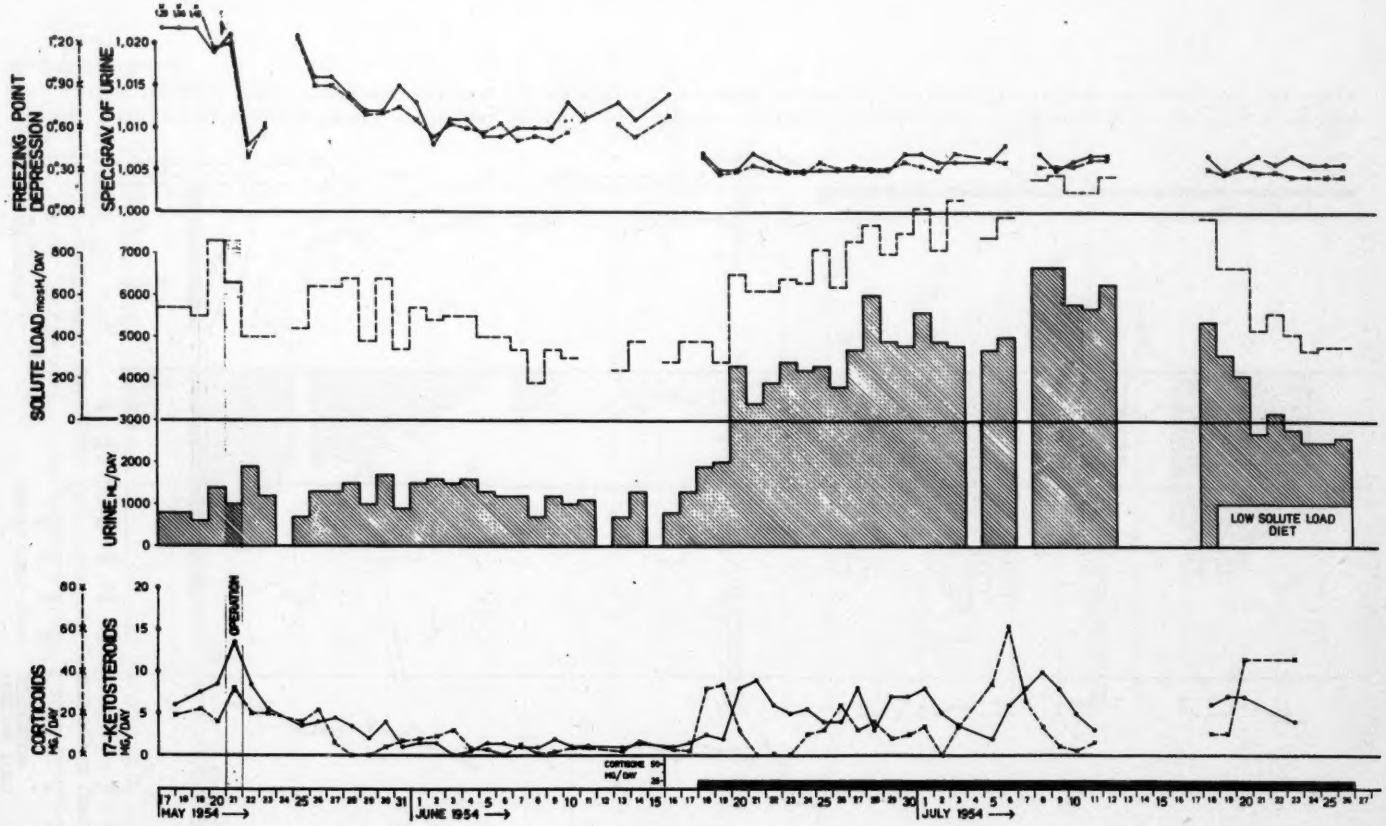

FIG. 4. Daily urine volume, effective solute load, excretion of 17-ketosteroids and corticoids, as well as changes in the specific gravity and osmolarity of the urine after hypophysectomy in a woman of 59 with metastatic cancer of the breast. No cortisone was given during the operation and for twenty-eight days afterwards.

postoperative days; and Group 3—cortisone or ACTH continuously.

The postoperative administration of cortisone or ACTH was of no significance in the production of these changes (Fig. 2). The only difference observed between the treated patients (Groups 2 and 3) and the untreated ones (Group 1) was a tendency to larger urine volumes during the second diuretic phase in the former.

The polyuria continued only in the patients getting cortisone continuously (Group 3, Fig. 2). The urine volume decreased after the second polyuric phase in the patients who either did not receive cortisone (Group 1, Fig. 2), or received it only for the first five postoperative days (Group 2, Fig. 2). In the latter two groups the diuresis during the fourth postoperative week amounted to about 1 liter per day. When cortisone was started in the patients of these groups, the polyuria reappeared (Figs. 3 and 4).

Thirst

An increased thirst was noted in all patients immediately after the operation. In Group 3 the thirst remained and was intense during the whole period of observation, whereas it decreased or disappeared in the two other groups before the maintenance dose of cortisone was started.

Excretion of a water load

The results of the oral water-load test before and after hypophysectomy are presented in Figure 6 and Table 1. The test during the postoperative period was performed after the patients had passed the second diuretic peak and cortisone had been omitted for at least one week. The lowest specific gravity, both before and after hypophysectomy, was observed in the second-hour urine sample, being 1.0052 ± 0.0004 and 1.0095 ± 0.0007, respectively. The difference was highly significant (P-value <0.001). The amount

TABLE 1. THE RESULTS OF THE 4-HOUR WATER-LOAD TEST BEFORE HYPOPHYSECTOMY AND DURING THE FOURTH POSTOPERATIVE WEEK, WHEN CORTISONE WAS NOT GIVEN

	Spec. gravity		Volume (4-hour)
	Night urine	Second-hour urine	
Before op.			
avge.	1.0183	1.0052	926
S.E.	0.0012	0.0004	41
After op.			
avge.	1.0100	1.0095	300
S.E.	0.0007	0.0007	37
P-value	<0.001	<0.001	<0.001

ation and for twenty-eight days afterwards.

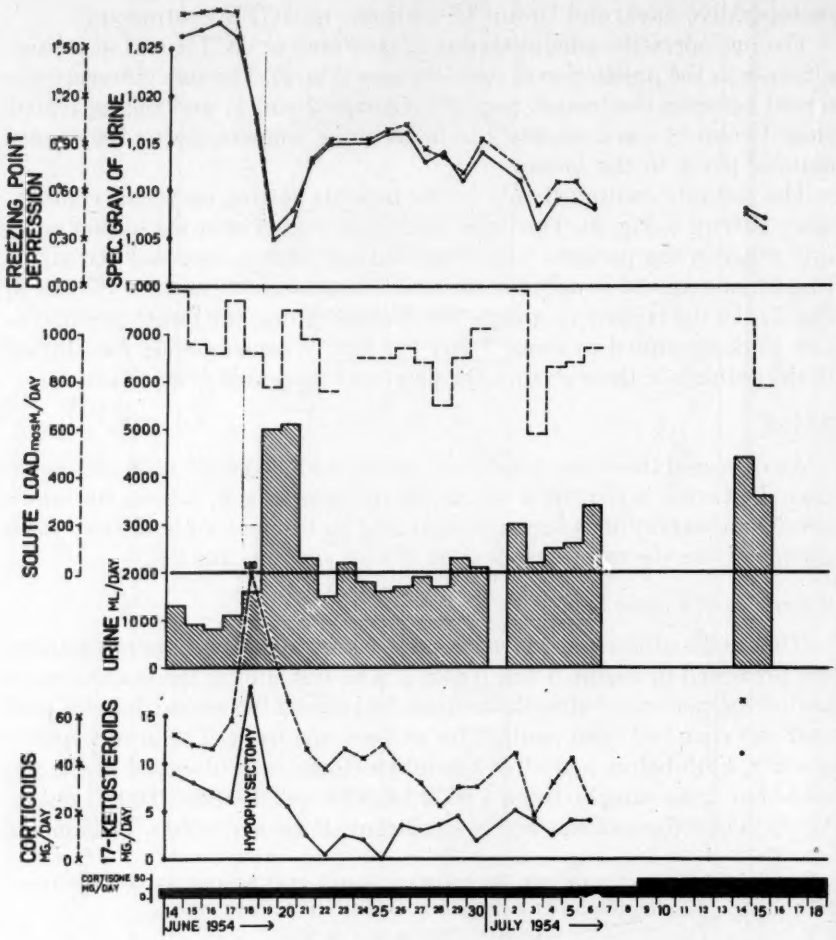

FIG. 5. Daily urine volume, effective solute load, excretion of 17-ketosteroids and corticoids, as well as changes in the specific gravity and osmolarity of the urine after hypophysectomy in a man of 59 with metastatic cancer of the prostate. Continuous administration of cortisone.

of urine excreted during the four hours was 926 ± 41 ml. before, and 300 ± 37 ml. after the operation, the difference being highly significant (P-value < 0.001). The night urine specimen was more concentrated before the operation than afterwards, the specific gravities being 1.018 ± 0.0012 and 1.010 ± 0.0007, respectively (P-value < 0.001). In 2 of the cases the administration of the water load intravenously did not change the results (Table 2).

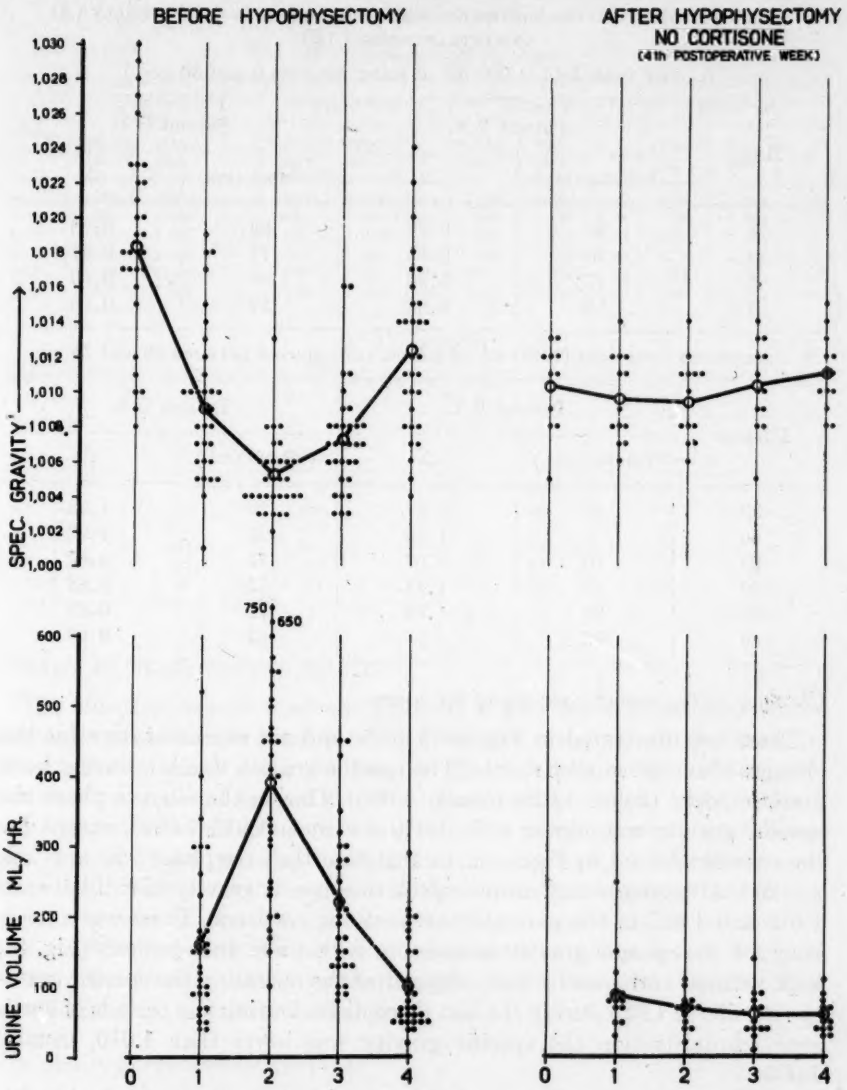

FIG. 6. The 4-hour water load test before hypophysectomy and during the fourth postoperative week, when cortisone was not given. ● denotes individual values; ○ denotes mean values.

TABLE 2. COMPARISON OF THE RESULTS OF A WATER-LOAD TEST IN HYPOPHYSECTOMIZED PATIENTS NOT RECEIVING CORTISONE, WHEN THE LOAD WAS GIVEN ORALLY (*A*) AND INTRAVENOUSLY (*B*)

A. *Oral water load* (1,000 ml. of water between 0 and 30 min.)

Hours	Patient V.A.		Patient G.L.	
	Volume (ml.)	Δ	Volume (ml.)	Δ
1	28	0.90	82	0.48
2	16	0.92	71	0.47
3	17	0.90	86	0.50
4	15	0.88	82	0.50

B. *Intravenous water load* (1,000 ml. of 5.5 per cent glucose between 30 and 75 min.)

Minutes	Patient V.A		Patient G.L.	
	Volume (ml.)	Δ	Volume (ml.)	Δ
30	15	1.27	24	1.02
60	8	1.30	25	1.02
90	10	0.97	72	0.83
120	15	1.17	73	0.88
150	37	1.16	72	0.89
180	15		62	0.90

Changes in the specific gravity of the urine

These are illustrated in Figures 3 to 5, and are representative for the changes observed in all patients. The specific gravity was low during both diuretic peaks (below 1.010, usually 1.005). During the oliguric phase the specific gravity was higher than 1.010 (between 1.015–1.022), except for the case illustrated in Figure 3. In this case the interphase was not well marked. After the second diuretic peak the specific gravity varied between 1.010 and 1.015 in the patients not receiving cortisone. There was a tendency for the specific gravity to increase with time. In 1 patient (Fig. 3), kept without cortisone for forty days after the operation the specific gravity rose above 1.020 during the last three days. During the periods of cortisone administration the specific gravity was lower than 1.010, usually 1.005.

The spontaneous variations of the concentration of the urine over a 24-hour period were followed in 2 patients not receiving cortisone and in 4 patients while taking 25 mg. of cortisone per day (Table 3). A dilute urine was excreted during the night hours in the patients not getting cortisone. No obvious differences over a 24-hour period were seen in the patients receiving cortisone.

TABLE 3. SPONTANEOUS DIURNAL VARIATIONS IN THE CONCENTRATION OF THE URINE AFTER HYPOPHYSECTOMY IN MAN

A. *With cortisone (25 mg./day)*

Hours	Patient G.L.			Patient G.J.			Patient M.S.			Patient L.S.		
	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ
8–12	250	1.011	—	370	1.010	0.54	950	1.007	0.35	1425	1.004	0.15
12–16	380	1.009	—	1320	1.005	0.30	1490	1.007	0.38	1720	1.004	0.17
16–20	465	1.007	—	620	1.008	0.45	865	1.007	0.34	1350	1.003	0.12
20–24	1560	1.007	—	445	1.007	0.42	490	1.008	0.42	1225	1.003	0.20
24–4	650	1.006	—	880	1.005	0.29	1105	1.005	0.34	970	1.003	0.17
4–8	495	1.007	—	700	1.008	0.50	1420	1.006	0.32	1010	1.005	0.25

B. *No cortisone*

Hours	Patient V.A.			Patient M.S.		
	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ
8–12			—	220	1.011	—
12–16	98	1.014	—	150	1.013	—
16–20	117	1.016	—	170	1.012	—
20–24	430	1.006	—	310	1.006	—
24–4	520	1.006	—	176	1.008	—
4–8	98	1.016	—	265	1.008	—

Changes in the effective osmotic load

The effective osmotic load was followed in 6 patients. Three representative cases are shown in Figures 3 to 5. The load progressively decreased in those cases in which cortisone was not given. A level of 200–300 mosM. per day was reached around the third postoperative week, and this level was maintained until cortisone was given. The administration of cortisone in a dose of 25 mg. per day brought about an increase in the effective osmotic load parallel to the increase in the diuresis and the decrease in the specific gravity of the urine. In 2 cases (Figs. 3 and 4) a low solute-load diet was given during the period of cortisone administration. A decrease in the urine volume parallel to the decrease in the load was obtained. The specific gravity remained unchanged.

The ability to concentrate the urine

A thirst test was performed during the fourth postoperative week in 2 patients before and after the cortisone administration was started. The test was also performed in the fourth postoperative week in 2 patients receiving cortisone continuously (Table 4). Except for a slight increase in Patient M. S., no increase in the specific gravity of the urine was observed during the water restriction, irrespective of cortisone administration.

TABLE 4. THE RESULTS OF THE 24-HOUR THIRST TEST AFTER HYPOPHYSECTOMY IN MAN

A. *With cortisone (25 mg./day)*

Hours	Patient M.S.			Patient G.L.			Patient G.J.			Patient L.S.		
	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ
8–12	370	1.010	0.52	215	1.012	0.68	360	1.008	0.56	580	1.005	0.25
12–16	680	1.008	0.47	168	1.012	0.73	860	1.008	0.45	400	1.008	0.32
16–20	590	1.008	0.47	248	1.012	0.68	720	1.008	0.45	388	1.007	0.50
20–24	380	1.010	0.64	140	1.011	0.65	385	1.009	0.60	350	1.006	0.41
24–4	146	1.012	0.76	442	1.001	0.60	220	1.012	0.81	175	1.009	0.37
4–8	200	1.015	0.92	338	1.012	0.68	—	—	—	225	1.010	0.56

B. *No cortisone*

Hours	Patient V.A.			Patient M.S.			Patient G.L.		
	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ	Volume (ml.)	Spec. grav.	Δ
8–12	37	—	1.10	132	1.013	0.76	105	1.015	0.75
12–16	62	—	1.09	168	1.013	0.79	208	1.016	0.81
16–20	85	—	1.01	182	1.013	0.78	280	1.012	0.64
20–24	73	—	1.00	125	1.014	0.79	221	1.013	0.64
24–4	70	—	1.12	113	1.014	0.88	507	1.014	0.72
4–8	70	—	1.10	121	1.015	0.89			

DISCUSSION

The results of the present study are discussed under the assumption that the subjects were completely hypophysectomized, *i.e.*, no functioning remnants of the hypophysis were present. The results of the tests used to establish the completeness of the hypophysectomy (6), as well as the follow-up study of the patients, support this assumption.

It is generally accepted that the ability of the kidney to produce a concentrated urine is dependent on the presence of antidiuretic hormone and a tubular epithelium capable of responding to this hormonal stimulus (7). It has been reported, however, that a concentrated urine might be excreted under conditions of extreme dehydration in animals with diabetes insipidus (8, 9, 10). Such a condition was not present in our series, and we therefore consider the excretion of a concentrated urine[1] in our patients as indicating the action of an antidiuretic factor on the tubular cells.

The over-all change in diuresis during the first two months, *i.e.*, a polyuria interrupted by a short period with lower urine volumes, is similar to the change observed in experimental diabetes insipidus in animals (8). In the animal experiments the oliguric phase has been attributed to the pres-

[1] A urine with a specific gravity or osmolarity higher than that of the deproteinized plasma.

ence of antidiuretic hormone produced in the degenerating cells of the posterior lobe of the hypophysis (8, 9), and it has been stated that a complete removal of the posterior lobe in animals invariably results in the immediate development of a permanent polyuria (11). Our results, however, indicate that in man the presence of a degenerating posterior lobe is not necessary for the appearance of the interphase.

The postoperative administration of ACTH or cortisone did not influence the appearance of the "normal interphase." The question whether adrenocortical steroids are of any significance in this respect cannot be answered, as even the patients not given cortisone were excreting significant amounts of corticoids during the interphase (Figs. 3 and 4).

Cortical steroids, however, were a prerequisite for the maintenance of the polyuria. After the third postoperative week the diuresis was large only if cortisone was given. It thus seems that one of the mediators of the diuretic action of the anterior lobe described in animals is the adrenocorticotropic hormone.

The mechanism by which cortisone increased the diuresis was twofold: 1) by a decrease in the osmolarity of the urine, which necessitated a larger amount of water for the excretion of a given solute load, and 2) by an increase in the daily load. This latter increase can be easily explained by the known metabolic effect of cortisone, as well as by the larger food intake of the patients taking cortisone.

The inability of the hypophysectomized patients not taking cortisone to excrete a water load within a normal period of time (four hours), and the absence of a lowering of the specific gravity of the urine during the water test, were obvious. This pathologic response was not due to a delayed or decreased intestinal absorption of water, as the administration of the load intravenously did not alter the response; nor was it due to an inability of the kidney to excrete a diluted urine. The diurnal variations of the specific gravity of the urine showed a low specific gravity—below 1.010—during the night hours. These findings are similar to those seen in adrenocortical insufficiency (3, 12). The action of cortisone on the excretion of the water load could not be evaluated, as cortisone induced a continuous polyuria with urine of a low specific gravity.

The presence of a concentrated urine after the oliguric phase in the hypophysectomized patients not receiving cortisone suggests the presence of antidiuretic activity under these conditions. It has been shown recently that antidiuretic activity can be demonstrated in the plasma of completely hypophysectomized rats, and that this activity increases after adrenalectomy (13). The site of production of antidiuretic hormone (if this is the antidiuretic factor) in our hypophysectomized patients could be the hypothalamus and/or the remaining part of the infundibulum.

The inability of these patients to excrete a concentrated urine while being treated with cortisone cannot be explained at present. Possible mechanisms to account for this defect during cortisone administration could be a decreased production or release of the antidiuretic hormone, an increased inactivation of the antidiuretic hormone, or a decreased ability of the tubular cells of hypophysectomized patients to respond to antidiuretic hormone.

The absence of a response to a 24-hour thirst test points to a defect in the control of the probable production and/or release of antidiuretic hormone during the first two months after hypophysectomy. This view is further supported by the absence of a normally concentrated urine in these patients.

SUMMARY

The changes in water excretion during the first two months after hypophysectomy, and the effect of cortisone upon these changes, were studied in 22 patients with metastatic cancer of the breast and 2 patients with metastatic cancer of the prostate.

Hypophysectomy induced a polyuria interrupted by a normal interphase which occurred about the end of the first week. The persistence of this polyuria after the third postoperative week was dependent on cortisone administration.

The mean specific gravity of the daily urine was below 1.010 during cortisone administration, and was increased when cortisone was withdrawn.

The response to a water load was abnormal when cortisone was not given. A 24-hour thirst test did not induce an increase in the specific gravity of the urine, whether cortisone was given or not.

The data are interpreted as showing: that the presence of the posterior lobe is not a prerequisite for the "normal interphase" in man; that one of the mediators of the diuretic action of the anterior lobe of the hypophysis is the adrenocorticotropic hormone; and that antidiuretic hormone is probably present in hypophysectomized patients but is produced and/or released in an abnormal fashion.

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THE TOLERANCE OF PATIENTS WITH MYXEDEMA FOR THYROID*

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THE myxedematous patient is said to be less tolerant than the normal subject to the administration of desiccated thyroid. Although this concept has long prevailed, objective support is meager. It has been suggested that the tolerance of the euthyroid patient derives from inactivation of the exogenous thyroid by the functioning thyroid gland (1, 2, 3). Others (4, 5) have observed in rat, dog and rabbit that thyroidectomy enhances the metabolic response to large doses of thyroid. The studies of Means and Lerman (6) indicate that the patient with myxedema has a striking initial response of the metabolic rate to desiccated thyroid, but that as the metabolic rate approaches normal, further response is comparable to that seen in normal subjects having similar metabolic rates. More recently Greer (7), after studying the radioiodide uptake of the human thyroid gland as influenced by administration of thyroid, suggested that the myxedematous and the euthyroid patient might be comparable in their responses, and that there is no inactivation of the administered thyroid by the gland. Riggs has recently expressed himself as being in complete agreement with Greer (8). Whether, in fact, there is any gross difference in response has been tested in the present study by the administration of desiccated thyroid to patients with myxedema in increasing doses until the point of drug-induced thyrotoxicity was reached.

MATERIAL AND METHODS

Patients with typical myxedema were given desiccated thyroid, beginning with a dose of 30 milligrams daily. The dose was increased at approximately fornightly intervals until the earliest evidences of drug-induced thyrotoxicosis were observed. The patients were observed for clinical response and, in addition, frequent determinations were made of the protein-bound iodine concentration of the serum (PBI), the basal metabolic rate

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(BMR), and body weight. Studies were terminated when clinical evidence of thyrotoxicosis developed.

Nine patients with myxedema were studied. Five had primary myxedema which had been present for periods of six to 240 months. In 4 patients the myxedema developed following surgery for thyrotoxicosis or for carcinoma of the thyroid gland. Myxedema had been present for from one to sixty months.

Three patients without myxedema were similarly studied. One of these had received 100 mg. of thyroid daily for hypothyroidism without myxedema (No. 10, Table 1). Another had a low metabolic rate and a low-normal PBI level (No. 11). Moderately severe exophthalmos had developed in the third (No. 12) after a transient attack of thyrotoxicosis several years previously. He was euthyroid on 150 mg. of thyroid daily at the beginning of the study.

RESULTS

An average daily dose of approximately 0.6 Gm. of desiccated thyroid was required to produce symptoms of thyrotoxicosis in the myxedema group, and an average of approximately 0.4 Gm. in the other 3 patients. Summaries of each individual study are found in Table 1. The early signs and symptoms of thyrotoxicosis appeared in association with approximately the same serum concentration of protein-bound iodine and the same degree of hypermetabolism in the two groups. The responses were entirely similar to those observed by others in normal subjects (1, 2, 9, 10). At the time thyrotoxicosis was evident, the basal metabolic rates averaged +10 per cent in the myxedema group, and +12 per cent in the others. The average serum protein-bound iodine concentrations were 9.6 and 11.2 micrograms per 100 ml., respectively, at the end of the studies. The patients with myxedema lost up to 16 per cent (mean 9.5 per cent) of their body weight during the study. The 3 patients without myxedema lost an average of 4 per cent of their body weight.

The signs and symptoms which indicated the development of thyrotoxicosis are listed in Table 1. In one of the myxedematous patients, anginal pain and minimal ankle edema developed for the first time during this study, and in another there was alarming hypoglycemia. One of the patients without myxedema had minimal ankle edema. Neither patient showing ankle edema was known to have heart disease; the edema disappeared when the dosage of thyroid was reduced.

The patterns of response of the non-myxedema group are illustrated in Figures 1 and 2. Patient No. 10 (Fig. 1) was hypometabolic at the onset and had been taking 100 mg. of thyroid daily for several years. During the course of the first three weeks of study, while the patient was taking 200

TABLE 1

Patient No.	Diagnosis	Age (yrs.)	Sex	At start of treatment			At time of induced thyrotoxicosis				Symptoms and signs of drug-induced thyrotoxicosis
				Weight (Kg.)	PBI (μg./100 ml.)	BMR (%)	Thyroid (Gm./day)	Weight (Kg.)	PBI (μg./100 ml.)	BMR %	
1	Primary myxedema	37	F	48.2	0.6	−43	1.80	42.4	14.7	+25	Lethargy, tachycardia
2	Primary myxedema	67	F	73.1	1.5	−37	0.59	64.2	11.7	+18	Tachycardia, excessive perspiration
3	Primary myxedema	63	M	85.4	0.2	−34	0.80	71.9	10.1	+16	Increasing fatigue, anorexia, tremor, tachycardia
4	Primary myxedema	72	F	63.6	1.4	−28	0.13	58.7	5.3	0	Nervousness, chest pain, ankle edema
5	Primary myxedema	42	F	77.7	1.5	−35	0.40	71.8	9.6*	+2	Palpitation, sense of fear
6	Postoperative myxedema	17	M	59.5	1.1	−68	0.40	51.3	10.9	+12*	Nervousness, weakness, tachycardia, excessive perspiration
7	Postoperative myxedema	50	F	68.4	0.5	−39	0.26	63.1	5.0*	0	Palpitation, transient exacerbation of previously asymptomatic ulcerative colitis
8	Postoperative myxedema	33	F	84.3	0.0	−35	0.59	78.5	9.7	+16	Nervousness, palpitation, tachycardia, excessive perspiration
9	Postoperative myxedema	29	M	69.0	0.8	−30	0.80	72.4	9.4	+4	Nervousness, tremor, tachycardia, excessive perspiration
10	Euthyroid	53	F	62.0	3.2	−24	0.50	61.7	9.8	+9	Palpitation, ankle edema
11	Euthyroid	18	F	57.2	4.2	−27	0.26	54.4	7.4*	+9	Nervousness, tachycardia, excessive perspiration
12	Euthyroid	43	M	54.1	8.0	+ 3	0.50	50.2	12.6	+19	Nervousness, palpitation, tremor, tachycardia

* These values were obtained shortly before toxicity was noted, rather than at the time toxicity appeared.

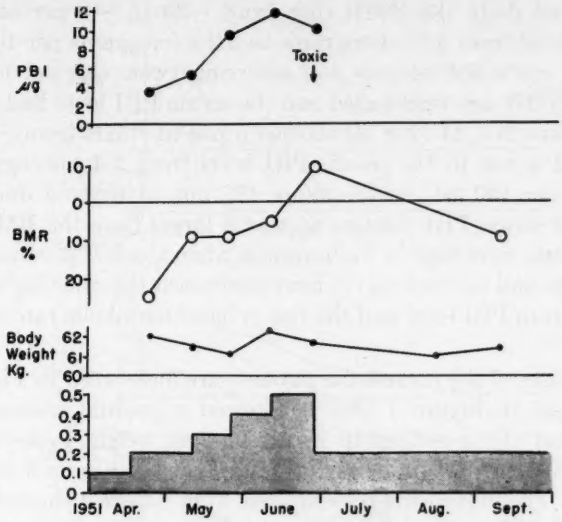

FIG. 1. The pattern of response to oral administration of increasing doses of thyroid in a patient (No. 9 of Table 1) who was on a maintenance dose of thyroid. Dosage in grams per day is indicated by the solid gray area.

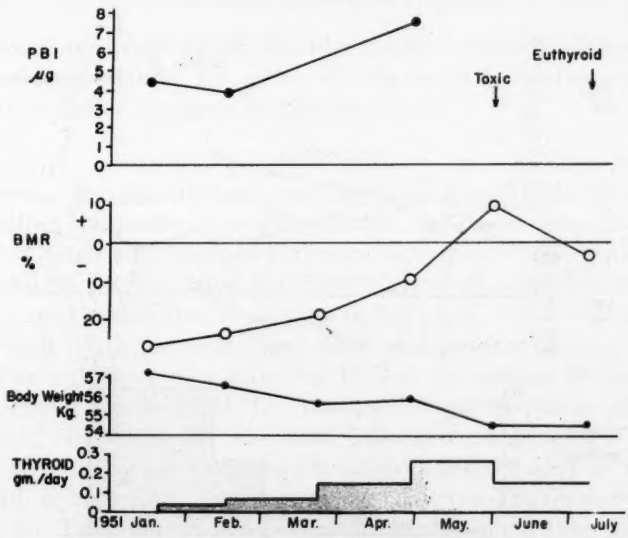

FIG. 2. Response to thyroid in a patient without myxedema but with hypometabolism (No. 11 of Table 1).

mg. of thyroid daily the BMR rose from −23 to −9 per cent and the serum PBI level from 3.9 micrograms to 5.2 micrograms per 100 ml. Four weeks later, while the patient was receiving twice the previous thyroid dosage, the BMR was unchanged and the serum PBI level had risen above normal. Patient No. 11 (Fig. 2) showed a rise in BMR from −25 to −10 per cent and a rise in the serum PBI level from 4.4 micrograms to 7.5 micrograms per 100 ml., while taking 130 mg. of thyroid daily. In both instances the serum PBI changes appeared larger than the BMR changes. The PBI values were high or high-normal when the BMR values were low-normal. Riggs and co-workers (2) have also noted this time lag between the rise in the serum PBI level and the rise in basal metabolic rate in euthyroid subjects.

The responses of the myxedema patients are illustrated in Figures 3 and 4. The patient in Figure 3 (No. 9) showed a gradual increase in BMR similar to that of the subject in Figure 2; their weight losses were also of similar magnitude. The final weight gain shown in Figure 3 was probably edema fluid. The myxedema patient (No. 3) in Figure 4 showed a response of BMR and PBI similar to that seen in Figure 1. Again there was an accompanying loss of weight. In Figures 3 and 4 there is a better correlation between BMR and serum PBI values than in Figures 1 and 2. The patient of Figure 3 was given thyroid in increasing doses after the first appearance

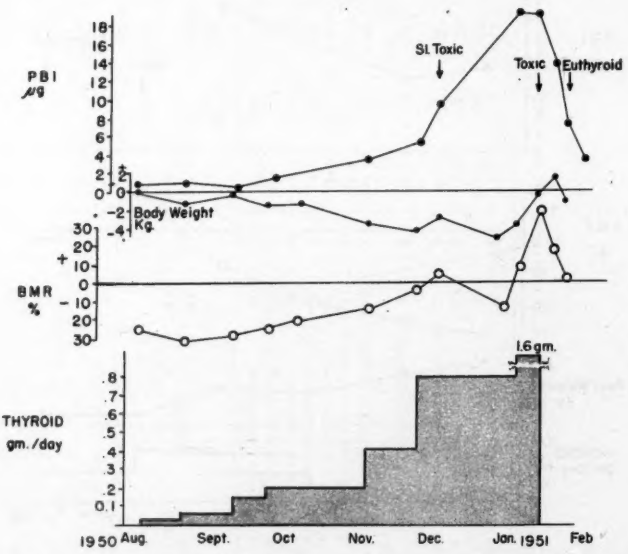

FIG. 3. Response of a patient with myxedema to thyroid (No. 9 of Table 1).

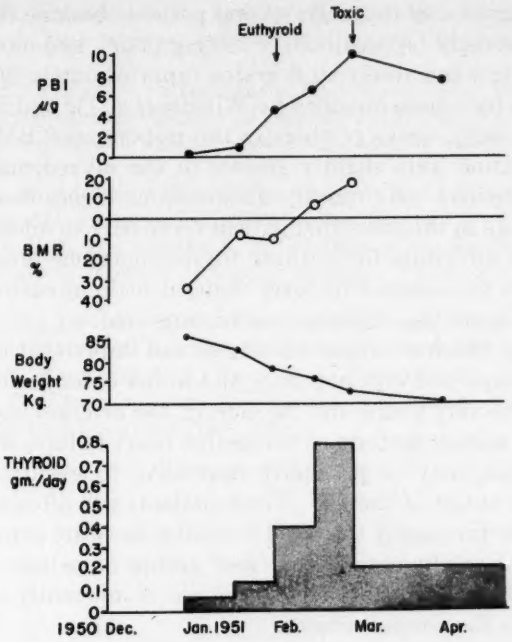

FIG. 4. Response of a patient with myxedema to thyroid (No. 3 of Table 1).

of symptoms of thyrotoxicosis, in order to observe the dose-response curve at higher dosage levels. He withstood the study in relative comfort but apparently modest retention of fluid developed.

COMMENT

Exogenous thyroid substance reduces the activity of the thyroid gland by inhibiting the production of thyrotropin by the anterior pituitary. In addition, Cortell and Rawson (11) have obtained evidence indicating a direct inhibitory effect upon the thyroid gland of administered thyroid. Not only are there anatomic changes in the gland, but functional alterations as well. With increasing oral doses of desiccated thyroid there is a progressive reduction in avidity for I^{131} by the normal (7) and by the iodine-deficient human gland (12). Doses which inhibit uptake of iodide are in excess of normal needs. The data presented here indicate that once normal needs are met, the tolerance to additional desiccated thyroid of patients with myxedema is similar to that of euthyroid subjects reported by others. Thus Farquharson and co-workers (9, 10) found that the response of normal subjects to thyroid was variable. In 1 of their patients thyrotoxicosis developed on 3 grains of thyroid daily (equivalent to approxi-

mately 200 mg. of USP thyroid); several patients became thyrotoxic on a dose of 4 grains daily (approximately 250 mg., USP), and most, but not all, were thyrotoxic when receiving 6 grains (approximately 400 mg. daily). Similar results have been obtained by Winkler *et al.* (1) and Riggs *et al.* (2).

During the early weeks of therapy the responses of BMR and serum PBI concentration were slightly greater in the myxedematous patients, but these differences were quickly dissipated at higher dosages. Presumably, small doses in the euthyroid patient serve only to inhibit the anterior pituitary, and substitute for intrinsic thyroid hormone production. Greer (6) found that the serum PBI level changed little in euthyroid subjects, until a dose of more than 130 mg. was administered.

The findings which are reported here do not imply that desiccated thyroid may be employed with impunity and in full doses in all patients with myxedema. The very young and the elderly, the arteriosclerotic, and those patients with angina pectoris or congestive heart failure, who in addition have myxedema, may be peculiarly responsive to even modest doses of thyroid at the outset of therapy. These patients will often adjust to doses which were not previously tolerated if smaller doses are employed initially. It may be that results obtained in these groups of patients have fostered the concept that the myxedematous patient is inherently less tolerant of thyroid than is the normal subject.

CONCLUSIONS

1. Administration of small doses of desiccated thyroid produced a greater measurable metabolic effect in 9 patients with myxedema than in 3 patients without myxedema, or in euthyroid subjects studied by others.

2. The tolerance to thyroid of the myxedematous patient after restoration of the euthyroid state is similar to that of the euthyroid patient.

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PSEUDO-PSEUDOHYPOPARATHYROIDISM:

REPORT OF A NEW CASE

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INTRODUCTION

THE syndrome of parathyroid deficiency (1) is characterized by chronic tetany, convulsive seizures, lenticular opacities, basal ganglia calcification, and low serum calcium and high serum phosphorus levels. Pseudohypoparathyroidism (2, 3) presents the same clinical and chemical picture presumed to be due to an inability of the organism to respond to parathyroid hormone, rather than to primary hormonal deficiency. In addition, characteristic abnormalities of body-build, facies and skeletal system, as well as foci of ectopic ossification are found in pseudo-hypoparathyroidism.

In 1952 Albright, Forbes and Henneman (4) reported a case of pseudohypoparathyroidism in which all of the usual anatomic stigmata were present, but the serum calcium and phosphorus levels were normal and the patient exhibited no clinical evidence of hypoparathyroidism. They called this new syndrome "pseudo-pseudohypoparathyroidism." In view of the absence of clinical and laboratory signs of hypoparathyroidism, one would expect a normal response to the Ellsworth-Howard test (phosphorus diuresis following intravenous parathyroid hormone injection). However, their patient did not respond normally to this test, possibly owing to the use of an impotent extract.

It is the purpose of this paper to report a new case of pseudo-pseudohypoparathyroidism (the second such case reported in the literature), in which the expected normal result of the Ellsworth-Howard test was observed, and to emphasize that certain abnormalities observed in this syndrome can occur independently of the hypoparathyroid state.

CASE REPORT

History: **Mrs. C. T.** (CGH No. 40218), a 24-year-old white, married nulliparous woman, was first seen at Colorado General Hospital in December 1951, because of amenorrhea for six months. Upon examination in the obstetrical clinic, there was no evidence of pregnancy. A complete physical examination was not made, but her height was recorded as 58 inches, and her weight as 187½ pounds. Roentgenograms showed the chest

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and skull to be normal. Because of obesity, hypothyroidism was considered, and basal metabolic rates were determined; the first was +11 per cent, and the second was +25 per cent. The serum cholesterol level was 187 mg. per 100 ml. Results of a "frog test" for pregnancy were negative.

The patient was not seen again for a year, until she came to Denver General Hospital in December 1952, complaining of pain in the right hand following a fall. X-ray examination showed a fracture of the proximal phalanx of the right fifth finger and anomalies of the metacarpals and phalanges of both hands. The finger was immobilized in a plaster splint, and the patient was urged to enter the hospital for study.

She returned to the Colorado General Hospital and was admitted from March 2 to March 9, 1953. Her only complaints were menstrual irregularities and vague pains in the back and both upper extremities.

Past history: She was born following an uncomplicated pregnancy. Her developmental history was normal so far as could be determined. However, neither the patient nor the patient's mother was sufficiently intelligent to provide adequate answers to many questions. At an early age she was sent to the Ridge Home for the mentally defective, where she remained until the age of 12. The patient stated that she completed the fifth grade there, but was not certain of this. There was no further formal education. She was sent

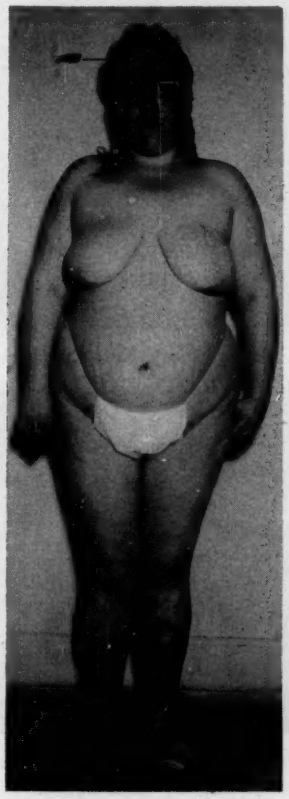

FIG. 1. Mrs. C. T. Note short stature, obesity and round face.

FIG. 2. Patient's hands. Note the short fingers.

FIG. 3. Patient's feet. Note the short toes.

FIG. 4. Patient's fists. Note the dimples over the metacarpophalangeal joints (Albright's "first sign").

to a number of schools, but the school physicians allegedly stated that she was more in need of medical care than education. A doctor in California made a diagnosis of rickets, but undertook no x-ray or blood studies. He recommended vitamin therapy, but for financial reasons this recommendation was not carried out.

Family history: The patient's mother was short and obese, but her fingers and toes were normal. The father was said to be short and obese, but he was not examined. The parents had been separated for many years. There were 8 half-siblings. One half-brother was said to have an appearance similar to that of the patient; his whereabouts were unknown.

Physical examination: Her height was 55 inches and weight 195 pounds. The face was round (Fig. 1). The heart was normal and blood pressure was 130/80. There were many purple striae over the entire abdomen and deformities of the fingers and toes (Figs. 2 and 3). With the formation of a fist, there was a dimpling of the skin over the metacarpal heads (Fig. 4). The left eye presented an inferior rectus paresis with hypermetropia and exotropia (paralytic). The patient gave a vague history of trauma to the eye and believed that the squint followed the trauma.

Laboratory data: Psychometric evaluation: verbal I.Q., 60; performance I.Q., 77; full scale I.Q., 65. Urinalysis: specific gravity 1.010 and 1.018; no albumin or reducing substances; no abnormalities in the sediment. Blood: hemoglobin 13.0 Gm.; erythrocyte count 4.4 million; leukocyte count 7,600, with a normal differential. Results of serologic examinations (Kolmer and Eagle) were negative on two occasions. Sulkowitch tests on the urine (March 3 and 6, 1953) showed "very fine precipitate" and "very slight amount of fine precipitate." Blood chemistry: plasma protein concentration 6.9 Gm. per cent; NPN 19 mg. per 100 ml.; chloride 99.6 mEq./L.; CO_2 67 vol. per cent; calcium 11.0 mg. per 100 ml. Unfortunately, no phosphorus determination was made. Urinary phosphorus: 0.24 Gm. per twenty-four hours.

Roentgenograms of the entire skeleton showed a mild coxa vara deformity (Fig. 5), in addition to the deformities of the metacarpals, metatarsals and phalanges (Fig. 6 and 7). All the long bones, vertebrae, ribs, pelvis and the skull appeared normal. No intracranial or subcutaneous calcifications were seen.

Subsequent course and findings: Upon the insistence of the patient's husband, she was discharged before studies were completed, but was readmitted from March 23 to April 1, 1953, for further investigation. Slit-lamp study of the lenses showed small peripheral opacities. Cervical and vaginal exfoliative smears were normal.

Additional laboratory determinations were as follows:

March 25: serum phosphorus level 5.4 mg. per 100 ml.; alkaline phosphatase 3.5 Bodansky units; acid phosphatase 1.2 units.

March 26: urinary calcium 0.11 Gm. and urinary phosphorus 2.1 Gm. per twenty-four hours.[1]

March 27: urinary calcium 0.07 Gm. and urinary phosphorus 1.6 Gm. per twenty-four hours.[1]

March 31: serum calcium level 10.3 and 10.6 mg. per 100 ml.

The BMR was +8 per cent. The electrocardiogram tracings were within normal limits, with a QT interval of 0.37 at a ventricular rate of 72. On March 27, 1953, the electroencephalogram was suggestive of a diffuse, moderately severe, cortical disturbance, possibly more marked in the left anterior temporal area.

On March 31, an Ellsworth-Howard test was performed. During the period of the

[1] The patient was receiving the regular hospital diet. We have no explanation for this increase in phosphorus excretion.

FIG. 5. Roentgenogram of hip, showing a mild coxa vara.

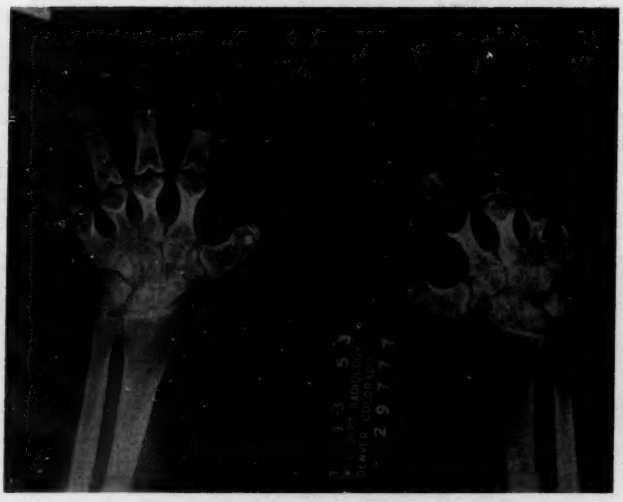

FIG. 6. Roentgenogram of the hands. Only the proximal phalanges of both thumbs and of the third finger, right, and the middle phalanges of the third and fourth fingers, left, are normal in length. All other metacarpals and phalanges are shortened. All of the involved phalanges have a deep AP groove across the middle of the base. The articulating condyles of adjacent metacarpals and phalanges are correspondingly irregular. The carpals appear normal. As a result of these deformities the hands are wide and stubby.

test the patient was fasting, but no attempt was made to regulate the fluid intake. The results were as follows:

Time	Urine volume (ml.)	Phosphorus (mg. per sample)
10–11 a.m.	2	2.1
11–12	32	15.4
12–1	88	30.8
	200 units Parathyroid Hormone (Lilly) i.v.	
1–2 p.m.	345	88.7
2–3	84	15.8*
3–4	380	99.9

* Low value may have been due to an incomplete collection. The patient was not catheterized.

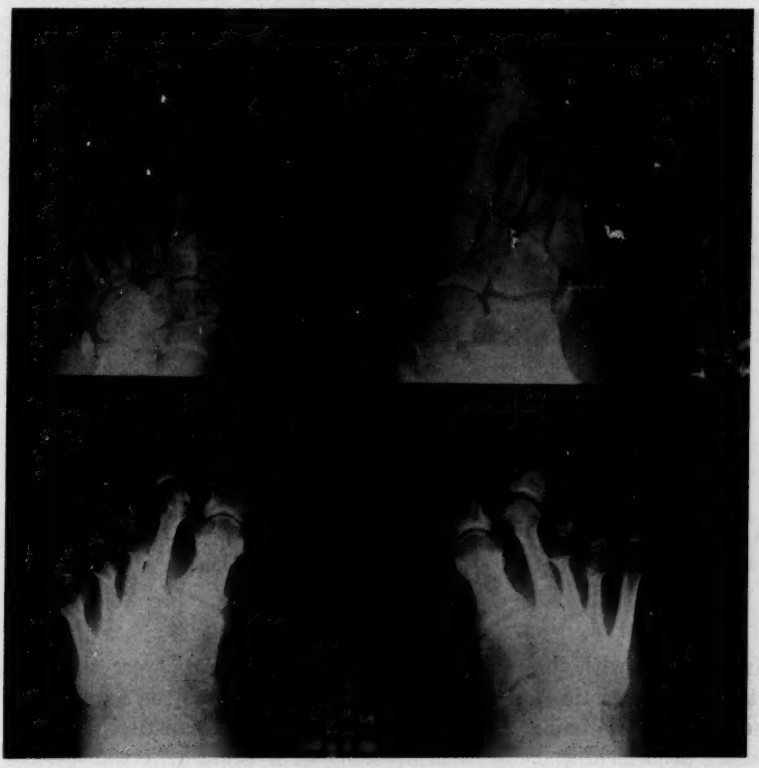

FIG. 7. Roentgenogram of the feet. There are deformities similar to those in the hands. The second metartarsal on the right, and the second and fifth metatarsals on the left are normal. All other metatarsals are shortened. Changes in the phalanges of the feet are much less marked than in the hands. No deep grooves are seen. The tarsals are grossly normal. Sesamoid bones in the tendons of the peroneus longus are prominent bilaterally.

Again the patient left the hospital against medical advice.

She returned October 6, 1953, because of concern over her amenorrhea. She was hospitalized for a week. She now gave a history of marked menstrual irregularities, which was quite different from the history she had given previously. She stated that her menarche occurred at the age of 15, but that she had had only one or two scanty menstrual periods a year. The last menses previous to this hospitalization had been approximately six months before.

On slit-lamp examination, the lens opacities were found to be progressing slowly. Roentgenograms of the skull again did not show calcification of the basal ganglia. Thyroidal radioiodine uptake on October 8, 1953, was 24.87 per cent in twenty-four hours, and excretion was 61.31 per cent in twenty-four hours. An endometrial biopsy showed rounded or dilated glands lined by occasional palisaded columnar cells. The glands were evenly distributed. This was thought to represent simple atrophy. Withdrawal bleeding occurred after a short course of stilbestrol, 1 mg. per day.

The electroencephalogram was interpreted as follows: "Moderately severe diffuse cortical disturbance similar to the test of March 27, 1953. However, something has been added since then, suggesting a progressive process of some type—a suppression over the left temporal area spreading up over the left motor strip. This could represent cortical damage in these areas, or an insulating type of process—overlying subdural hematoma, areas of calcification, etc.,—or both."

Repeated blood determinations were performed with the following results:

Date 1935	Ca (mg./100 ml.)	P* (mg./ 100 ml.)	Chol. (mg./ 100 ml.)	Alk. phospha-tase (B.U.)	NPN (mg./ 100 ml.)	Tot. protein (Gm. %)	Miscellaneous
Oct. 7	11.2	4.9	183	5.8	33	6.5	Glucose, 88 mg./100 ml.
Oct. 8	9.6	4.6	313	4.4	43	6.0	CO_2, 63 vol. %
Oct. 9		4.9		4.9			Uric acid, 3.6 mg./100 ml.
Oct. 10	12.0	4.8					
Oct. 12	11.2	4.5	217	5.7			
Oct. 13	12.3	5.0		5.8	28	6.6	Albumin 4.34 Gm. % Globulin 2.26 Gm. %

* The slightly elevated phosphorus values may have been secondary to hypoestrinism (*cf.* amenorrhea; and atrophy of endometrium in biopsy specimen).

DISCUSSION

In common with the case reported by Albright *et al.* were the presence of the characteristic skeletal abnormalities, body habitus and facies, the absence of basal ganglia calcification, the menstrual irregularities, and the absence of clinical and chemical evidence of hypoparathyroidism. On the other hand, the subcutaneous calcification and hypertension present in the original case were not observed. An additional finding, not previously reported in either pseudohypoparathyroidism or pseudo-pseudohypoparathyroidism, was the mild coxa vara.

Of interest from the genetic standpoint is the occurrence of lens opacities,

mental deficiency and diffuse electroencephalographic abnormalities. These are frequent findings in pseudo-hypoparathyroidism and idiopathic hypoparathyroidism and have often been considered secondary manifestations of the characteristic hypocalcemia. Their presence in our patient, whose serum calcium level was consistently normal or slightly above normal, is strong evidence that these abnormalities are due to a genetic defect which is independent of the hypoparathyroid state.

One of the first effects of parathyroid hormone injection is a phosphorus diuresis. This is the basis of the Ellsworth-Howard test (5). Normal individuals and patients with idiopathic hypoparathyroidism show a significant rise in urinary phosphorus during the three hours immediately following the intravenous administration of parathyroid hormone, whereas patients with pseudohypoparathyroidism do not (Fig. 8). Our patient showed a normal response to this test, as would be expected in view of the absence of all signs of parathyroid deficiency.

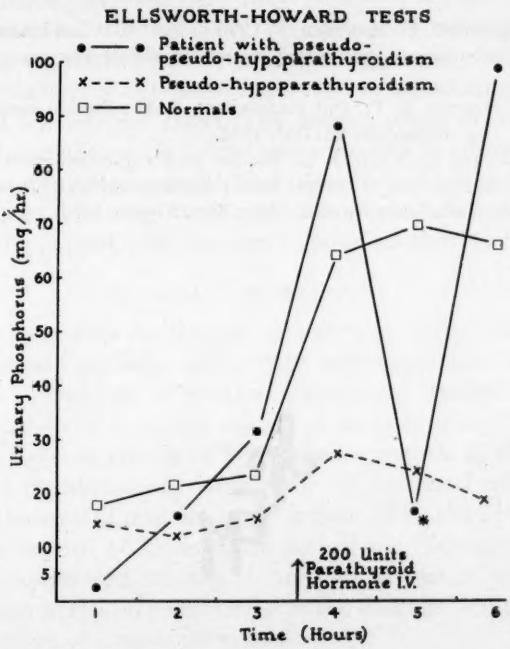

FIG. 8. Results of Ellsworth-Howard test in patient compared with those in normal subjects and in pseudohypoparathyroidism. Data for the latter curves were taken from the paper by Elrick *et al.* (3); the curves represent average values from 7 normal subjects and 6 patients with pseudohypoparathyroidism, respectively.

* This low value was associated with a very small urine sample, which suggests that the collection was incomplete.

SUMMARY

A new case of pseudo-pseudohypoparathyroidism is reported, the second described in the literature. A normal phosphorus diuresis followed injection of parathyroid hormone. Lens opacities, mental deficiency and electroencephalographic changes were present despite the absence of hypoparathyroidism. The significance of these findings is discussed briefly.

Acknowledgment

We are indebted to Dr. Raymond R. Lanier, Professor of Radiology, University of Colorado Medical School, for the interpretation of the x-ray films.

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EFFECT OF THYROTROPIN ON ADRENOCORTICAL FUNCTION*

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THIS study is concerned with the effect of thryrotropin administration on adrenocortical function in the hypothyroid and in the euthyroid subject. As reported previously (1) evidences of decreased adrenocortical function are encountered not only in instances of hypothyroidism secondary to adenohypophysial disease but also in patients or experimental animals with primary thyroidal deficiency. In the former group the administration of desiccated thyroid may precipitate adrenocortical insufficiency. In the latter subjects, however, following therapy with desiccated thyroid, various indices of adrenocortical function reflect increased physiologic activity of the adrenal gland. The present study demonstrates the effect of thyroid stimulation on the urinary excretion of adrenal steroids in subjects with panhypopituitarism. These observations are contrasted with the results obtained following thyrotropin administration to the euthyroid subject and to the patient with primary hypothyroidism.

MATERIAL AND METHODS

The investigations were conducted on 2 subjects with euthyroidism, 3 with primary hypothyroidism, and 4 with hypothyroidism secondary to hypopituitarism. Following a control observation period, thyrotropin (TSH) was administered in a daily dosage of 25 to 50 mg. (10 to 20 international units) in one or two daily injections for periods of six to twenty-one days. Serial measurements were made of the basal metabolic rate (BMR), the serum level of protein-bound iodine (SPI), the 24-hour urinary excretion of the neutral 17-ketosteroids and of the "formaldehydogenic" corticoids ("11-oxygenated steroids"), and the degree of exophthalmos. Serial skin biopsies were also performed and the findings in the latter study will form the subject of a separate report.

The basal metabolic rate was determined in the conventional fashion. The urinary excretion of the neutral 17-ketosteroids was measured by means of a modification of the method of Callow, Callow and Emmens (2);

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that of the "formaldehydogenic" corticoids by a modification of the method of Corcoran and Page (3). The serum protein-bound iodine concentration was estimated according to the method of Barker, Humphrey and Soley (4). Exophthalmometric readings were made with a Hertel exophthalmometer.

RESULTS

The results are summarized in Table 1.

Euthyroidism

In the 2 euthyroid subjects the administration of thyrotropin was followed by a rise in the basal metabolic rate and the level of serum protein-bound iodine, unattended, however, by any of the manifestations of thyrotoxicosis (Fig. 1). In both subjects the 24-hour urinary excretion of the "formaldehydogenic" corticoids decreased. The urinary excretion of the neutral 17-ketosteroids remained unaltered in one subject and decreased in the other. In the former, the exophthalmometric readings appeared to increase during treatment, whereas in the latter a slight decrease was noted following cessation of therapy.

TABLE 1

Diagnosis	BMR (%)	SPI (γ/100 ml.)	FG* corticoids (mg./24 hrs.)	Neut. 17-KS (mg./24 hrs.)	Exoph. reading (mm.) O.S.	Exoph. reading (mm.) O.D.	TSH therapy (mg. ×days)
I. *Hypopituitarism*							
1) C.S. (Sheehan's syndrome)	Pre −29	1.5	0.4	1.0	16	16	25 mg. ×6
	Post −17 (after crisis)		0	0.8			
2) V.E. (Sheehan's syndrome)	Pre −27	4.2	0.8	0.7			25 mg. ×8
	Post −17	7.4	0.0	0.8			
3) M.V. (Sheehan's syndrome)	Pre −21	1.4	0.15	0.9	11	10	25 mg. ×11
	Post − 4	5.4	0.0	0.8	13	12	
II. *Primary hypothyroidism*							
1) R.R. (after thyroidectomy for Hashimoto struma)	Pre −14	3.9	0.7	1.9	22	22	25 mg. ×9
	Post −26	3.2	1.7	3.5	23	22.5	
2) T.G. (juvenile myxedema)	Pre −13	1.7	0.6	3.0	16	17	50 mg. ×10
	Post −10	1.7	0.7	1.5	17	17	
3) E.W. (after thyroidectomy for Graves' disease)	Pre −35	3.1	0.4	5.6	23	23	25 mg. ×7
	Post −36	3.6	0.4	5.1	22	22	
III. *Euthyroidism*							
1) T.R.	Pre − 7	2.4	2.0	7.0	15	15	25 mg. ×7
	Post +13	14.0	0.7	5.2	17	16	
2) M.B.	Pre + 7	6.0	1.7	7.0	18	18	50 mg. ×7
	Post +55	26.2	0.3	1.4	18	18	25 mg. ×1

* FG = formaldehydogenic.

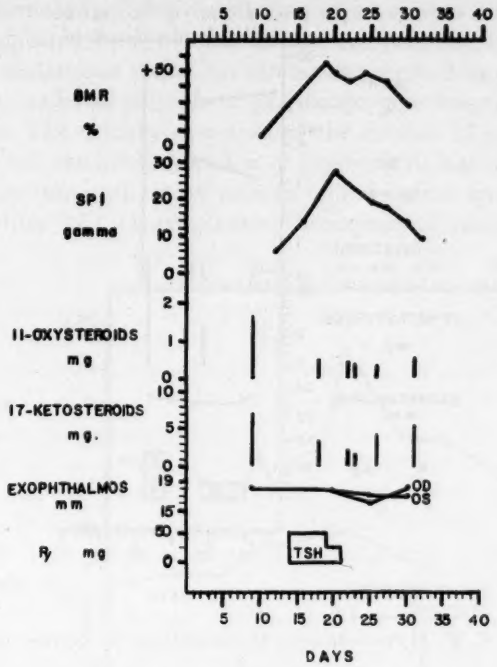

FIG. 1. *M. B.* Euthyroid.

Primary hypothyroidism

In all 3 subjects with primary hypothyroidism the administration of thyrotropin was without effect on the basal metabolic rate, the serum level of protein-bound iodine, the degree of exophthalmos, or the urinary excretion of the neutral 17-ketosteroids (Fig. 2). The urinary excretion of the "formaldehydogenic" corticoids remained unaltered in 2 subjects, but in the third patient an increase was noted following treatment with thyrotropin. In all subjects, the administration of corticotropin (ACTH) was followed by an increase in the urinary excretion of the adrenocortical steroid fractions, indicating the ability of the adrenal cortex to respond to stimulation by corticotropin.

Hypopituitarism

In the 3 subjects with Sheehan's syndrome studied, the administration of thyrotropin was followed by an increase in the basal metabolic rate and /or serum protein-bound iodine level, improvement in the symptoms of hypothyroidism, and disappearance of the urinary "formaldehydogenic"

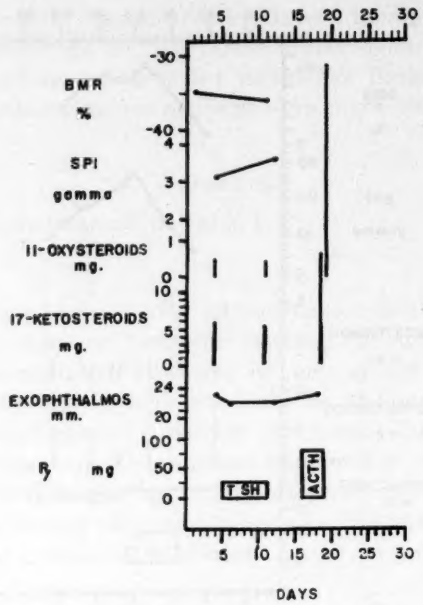

FIG. 2. *E. W.* Myxedema after thyroidectomy for Graves' disease.

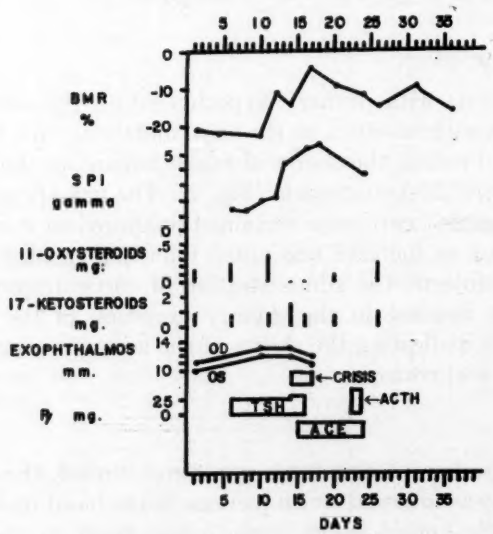

FIG. 3. *M. V.* Sheehan's syndrome—Hypopituitarism.

corticoids concomitant with precipitation of an adrenocortical crisis. The crisis was manifested by symptoms of nausea, vomiting, fever, anorexia, asthenia and abdominal pain. No alterations were demonstrable in the levels of serum sodium, chloride or potassium, nor was there any fall in blood pressure. The urinary excretion of the neutral 17-ketosteroids was unchanged. When the administration of thyrotropin was discontinued the basal metabolic rate and serum protein-bound iodine level fell, and the urinary excretion of "11-oxygenated" compounds increased (Fig. 3).

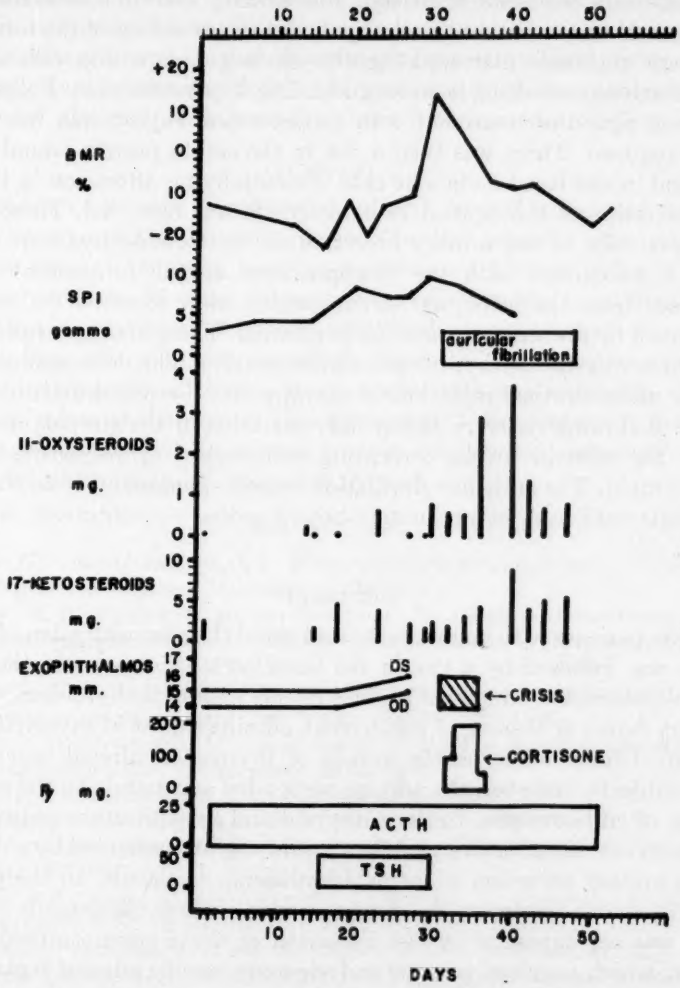

FIG. 4. *E. M.* Hypopituitarism—Chromophobe adenoma.

Adequate data on the degree of exophthalmos were available in only 1 subject, in whom a 2-mm. increase in the exophthalmometric reading was noted following the administration of thyrotropin.

An attempt was made to measure the effectiveness of thyrotropin in inducing adrenocortical crisis in a patient with hypopituitarism receiving a fixed amount of corticotropin (Fig. 4). Subject E. M., a patient with a chromophobe adenoma, was given 25 mg. of corticotropin daily. During this regimen the serum protein-bound iodine concentration and basal metabolic rate remained unaltered. The urinary excretion of neutral 17-ketosteroids was essentially unchanged, and the excretion of the formaldehydogenic corticoids increased slightly—findings compatible with adrenocortical atrophy resulting from long-standing hypopituitarism. Following a two-week period of treatment with corticotropin, thyrotropin was added to the regimen. There was then a rise in the serum protein-bound iodine level and in the basal metabolic rate. Essentially no alteration in the urinary excretion of the neutral 17-ketosteroids was observed. There was a temporary rise in the urinary excretion of the formaldehydogenic corticoids. Concomitant with the disappearance of the formaldehydogenic corticoids from the urine, an adrenocortical crisis occurred and was accompanied by the onset of auricular fibrillation. These findings appeared on the fourteenth day of thyrotropin administration. The crisis responded to specific adrenocortical replacement therapy and the withdrawal of thyrotropin. Following recovery the urinary excretion of the steroids remained above the control levels, indicating adrenocortical responsiveness to corticotropin. The auricular fibrillation ceased spontaneously as the basal metabolic rate and serum protein-bound iodine concentration were reduced.

DISCUSSION

In the patients with an intact thyroid gland the administration of thyrotropin was followed by a rise in the basal metabolic rate and the serum level of protein-bound iodine. In the subjects with hypothyroidism due to a primary defect or absence of the thyroid, administration of thyrotropin was without definite effect on the indices of thyroid or adrenal function. In these subjects, however, the adrenal responded adequately to the administration of corticotropin. In the euthyroid and hypopituitary patients, the increased functional activity of the thyroid was accompanied by a decrease in the urinary excretion of formaldehydogenic corticoids. In the patients with Sheehan's syndrome the disappearance of these compounds from the urine was accompanied by the induction of acute adrenocortical insufficiency, which required prompt and vigorous specific adrenal replacement therapy. It is of interest to note that the patients with hypopituitarism and in 1 of the 2 euthyroid subjects, the decrease in the urinary excretion of the

formaldehydogenic corticoids was not associated with any changes in the urinary excretion of the neutral 17-ketosteroids. In the patients with hypopituitarism treated with thyrotropin, the precipitation of a state of adrenal insufficiency in association with the disappearance of the formaldehydogenic corticoids in the urine would suggest that physiologic need for adrenocortical hormones of the glycogenic corticoid type exceeds the capacity of the adrenal to produce them when the metabolic rate is increased.

The decreased urinary excretion of neutral 17-ketosteroids often observed in subjects with thyrotoxicosis may reflect the malnutrition and wasting often observed in that disorder. On the other hand, in such patients the urinary excretion of the "11 oxycorticoids" is frequently normal or increased, although some low values have been encountered (5–8).

SUMMARY

Thyrotropin has been administered to subjects with euthyroidism and primary and secondary hypothyroidism. In the subjects with primary hypofunction of the thyroid, no effect was demonstrated on either thyroid or adrenal function. In other subjects, an increase in the basal metabolic rate and in the serum level of protein-bound iodine was observed. Concomitant with these changes there was noted a decrease in the urinary excretion of formaldehydogenic corticoids, unassociated with any alteration of the urinary excretion of the neutral 17-ketosteroids. These findings help to explain the precipitation of an adrenal crisis in subjects with Sheehan's syndrome given thyrotropin or desiccated thyroid.

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THE THYROIDAL UPTAKE OF RADIOACTIVE IODINE AS MODIFIED BY AN IODINE-RESTRICTED DIET*

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A LOW thyroidal uptake of radioactive iodine (I^{131}), in the absence of clinical hypothyroidism is not uncommonly noted. Low uptakes can frequently be related to exogenous thyroid medication, to recently administered antithyroid drugs, or to recently administered I^{127} in the form of medication or in contrast x-ray media. Patients with thyroid disorders often receive one of the foregoing substances, and the inquiring physician will look for a history of such medication when confronted with an unexpectedly low thyroidal uptake of I^{131}.

A high uptake of I^{131}, on the other hand, is much less frequently encountered in the absence of hyperthyroidism, but the reasons for a high uptake may be much more obscure. Thus, without critical clinical and laboratory evaluation, a high uptake of I^{131} may lead to an erroneous diagnosis.

High uptakes in the absence of hyperthyroidism have been noted by various investigators. Stanbury *et al.* (1) found abnormally high uptakes of I^{131} in goitrous individuals in the endemic goiter area of Mendoza, Argentina. Guinet and Berger (2) noted high I^{131} uptakes in young patients with "juvenile goiter" who were euthyroid by clinical and other laboratory standards, and who were apparently not iodine deficient. An increased thyroidal avidity for iodine was found by McGirr and Hutchison (3) and Stanbury and Hedge (4) in certain cases of nonendemic goitrous cretinism. Klotz and Lumbroso (5) reported 5 euthyroid patients in whom the I^{131} uptake was high. Two of these cases represented continued high uptakes in previously thyrotoxic patients whose metabolic state had clinically returned to normal following I^{131} therapy. This last phenomenon has been occasionally observed by us and has usually been transient. It may repre-

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† U. S. Public Health Trainee in the National Institute of Arthritis and Metabolic Diseases.

sent a "recovery" phase, during which the thyroid gland which has depleted itself of iodine through overactivity has a transient period of increased avidity for iodine.

There are a few euthyroid patients with high I^{131} uptakes who do not fall into any of the previously described groups. They are rare, and each must be considered individually.

A large number of patients on the Kempner rice regimen (6) are seen at Duke Hospital. It became apparent that many of them showed a high thyroidal uptake of I^{131}, although there was no clinical or laboratory evidence of thyrotoxicosis. Actually, hypothyroidism was suspected in several instances. To investigate this phenomenon, we studied a series of patients who had been on the rice regimen for varying lengths of time, and in whom no subjective or objective evidence of hyperthyroidism was evident.

PROCEDURE

Twenty patients who were receiving the rice diet are included in this group. The duration of this diet ranged between four days and eight years. The serum cholesterol level was routinely estimated in each patient and, when there was the slightest indication of thyroid hyperactivity, basal metabolic rates were obtained.

In determining I^{131} uptake, a tracer dose of 20 microcuries of I^{131} was given, and twenty-four hours later the percentage present in the thyroid gland was noted. All measurements were made with an Aloe scintillation counter, model NRD-SC3.

The results obtained in this group were compared with I^{131} uptakes obtained in 125 euthyroid patients not receiving the rice diet. All determinations were made during the same nine-month period and using the same technique. Patients were classified as euthyroid on the basis of our final analysis of all laboratory and clinical findings. The normal range of thyroidal I^{131} uptake at this laboratory is considered to be 15 to 45 per cent, inclusive.

RESULTS

Controls: Of 125 euthyroid patients not receiving the rice diet (Fig. 1A), 115 (92 per cent) had I^{131} uptakes within the normal range. Eight (6.4 per cent) had uptakes of 14 per cent or less. Two (1.6 per cent) had uptakes of 46 per cent or more. One of the 2 euthyroid patients with a high uptake was a young girl from an endemic goiter area, who was thought to have an iodine-deficient gland. There was no explanation for the high uptake in the second patient.

Rice-diet patients: The findings in 20 euthyroid rice-diet patients are summarized in Table 1. Of the 20 patients, 10 (50 per cent) had I^{131} uptakes

of 46 per cent or more (Fig. 1B). There were 12 patients who had been on the diet for two years or more. Of these, 9 (75 per cent) had uptakes of 46 per cent or more (Fig. 1C). Two of the remaining 3 patients had uptakes of over 40 per cent. Of 8 patients who had been on the rice diet for three months or less, only 1 (12.5 per cent) had a thyroidal I^{131} uptake of greater than 45 per cent (Fig. 1D).

DISCUSSION

These data show there is increased avidity of the thyroid gland for iodine in many patients on the rice diet. This increased I^{131} uptake appears to develop after a prolonged period on the diet—in this study, two years—

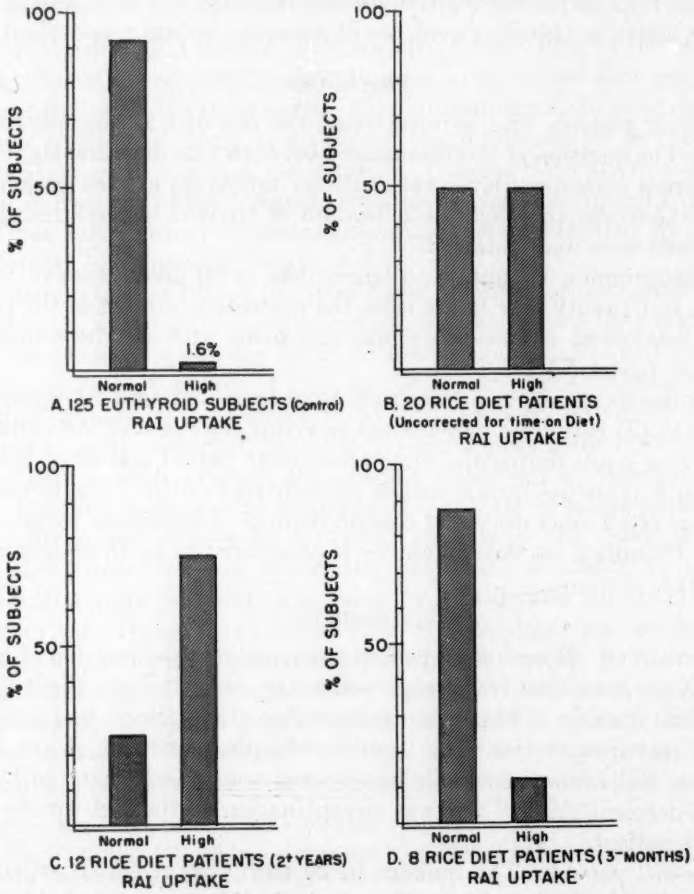

FIGURE 1

TABLE 1

Patient (record No., age, sex)	Diagnosis	B. M. R. %	Serum cholesterol (mg./100 ml.)	24-Hour I^{131} uptake (%)	Time on rice diet
1. E.R. B96204 49WF	Hypertensive arteriosclerotic cardiovascular disease; failure	+30*	225	85	8 yrs.
2. J.W. B71843 34WM	Hypertensive vascular disease	—	174	92	6 yrs.
3. O.F. C29156 69WF	Hypertensive cardiovascular disease	—	263	56	6 yrs.
4. A.B. C52158 57WF	Hypertensive cardiovascular-renal disease	+22	313	67	5 yrs.
5. W.T. C19444 64WM	Arteriosclerotic heart disease; coronary insufficiency	—	259	44	5 yrs.
6. F.V. B73286 35WM	Hypertensive vascular disease	—	190	91	4½ yrs.
7. H.P. C80718 55WM	Hypertensive cardiovascular disease	—	360	79	3½ yrs.
8. O.R. D15535 56WM	Hypertensive cardiovascular-renal disease	—	203	43	3 yrs.
9. A.W. D17279 55WM	Hypertensive cardiovascular-renal disease	—	237	62	3 yrs.
10. W.D. B98713 23WM	Chronic nephritis	−21	179	70	3 yrs.
11. P.E. D16878 56WM	Hypertensive cardiovascular disease	−21	270	38	2½ yrs.
12. C.N. D43600 23WF	Chronic glomerulonephritis	−5	163	72	2 yrs.
13. M.W. D31488 62WF	Hypertensive cardiovascular disease	−18	367	24	3 mos.
14. L.P. D79784 70WM	Hypertensive cardiovascular disease	—	374	15	3 mos.
15. J.R. D79283 53WM	Arteriosclerotic heart disease, coronary insufficiency	—	263	46	3 mos.
16. J.N. D80692 54WF	Hypertensive cardiovascular disease; diabetes	—	286	39	2 mos.
17. K.T. D83046 55WM	Hypertensive cardiovascular disease	—	302	19	2 mos.
18. N.G. D75580 35WF	Hypertensive vascular disease. Small nontoxic diffuse goiter	−1	274	18	1 wk.
19. E.B. C56506 43WF	Hypertensive cardiovascular disease. Nontoxic thyroid adenoma	−4	250	26	1 wk.
20. E.G. D83769 49WM	Hypertensive cardiovascular-renal disease; coronary insufficiency	+32	343	39	4 days

* Doubtful.

and does not appear in patients who were on the rice diet for three months or less. From present statistics, it is not possible to say just when this change takes place. No patients were examined between three months and a year.

The common denominator of this group of patients is the rice diet. Apparently something in the nature of the diet causes the increased uptake of I^{131}. Usually no drugs are given to these patients. That the increase in I^{131} uptake takes place only after a prolonged period of time on the diet suggests that a deficiency phenomenon is the factor.

Patients on the rice regimen, as managed in this clinic, are usually kept on a "basic" diet of rice, fruit (without salt or preservatives), sugar and honey for three months or longer while being observed. At the end of this time, depending upon the patient's response and condition, certain small quantities of additional food are allowed. This modification consists of such additions as 2 to 14 ounces of lean meat per week, 2 to 6 ounces of potato a day, and similar small amounts of vegetables and legumes. The modifica-

tions vary considerably from patient to patient, never being other than small, and the allowances are not necessarily always eaten. In this study, the patients were almost religious in their adherence to the rice diet, and their faithfulness was regularly checked by estimations of the urinary chlorides.

The iodine content of a specific foodstuff varies widely, depending upon the area of the world in which it is produced and upon the laboratory in which the content is determined (7). This fact, together with the variation in modifications of the diets of these patients, made impossible an accurate estimation of the usual daily consumption of iodine of each patient in this series. One day a patient might eat tomatoes grown in Indiana; the next, celery shipped from Florida. Using the accumulated data of various laboratories (7), a rather gross estimation of the available iodine in the diet of the usual patient on the "basic" and "modified" rice diets could, however, be made. This was about 8 to 10 micrograms per day for the "basic" diet and 20 to 25 micrograms per day for the usual "modified" diet.

The minimum daily requirement for iodine (MDR) has never been definitely established, largely due to technical difficulties. Estimates vary between 14 and 25 micrograms per day (8, 9). Stanbury *et al.* (1) gave estimates of 15 to 40 micrograms per day. These variations in estimation further point out the difficulty of study and measurement. However by any standard, the MRD is small.

The estimate of 8 to 10 micrograms of iodine per day for the "basic" diet is certainly below the lowest estimate of minimum daily iodine requirement, and 20 to 25 micrograms per day for the "modified" diet is, at least, marginal. It is believed, therefore, that the high I^{131} uptakes found frequently in euthyroid patients receiving the rice diet for prolonged periods are a result of a restricted iodine intake. That the uptake should become elevated only after more than three months on the diet may be explained by the ability of the body to store iodine, as demonstrated by Marine (10). There must be strict adherence to the rice diet in order to produce this increased I^{131} uptake. An occasional salting of the food could easily prevent this phenomenon from developing, as in each gram of iodized table salt there is an amount of iodine (160 micrograms), which constitutes at least 4 MDR.

It is of interest that only 2 of the 12 patients who had some modification of their diets admitted eating fish or other seafood on other than extremely rare occasions. The one with a normal I^{131} uptake ate ocean seafood, and the other, with a 92 per cent uptake, ate fresh water fish. Fresh water fish, unlike salt water fish, is not unusually high in iodine content (12). In none of the 20 patients did thyroid enlargement develop while they were on the rice diet, though 2 patients with normal I^{131} uptakes had small goiters be-

fore starting the diet. Perhaps this was because the diet, as modified, did contain a marginal amount of iodine—insufficient to satisfy the gland's avidity for iodine, but enough to prevent compensatory enlargement. No evidence of nutritional deficiency was observed in any patient.

SUMMARY AND CONCLUSIONS

1. A high uptake of radioactive iodine (I^{131}) by the thyroid gland is frequently found in euthyroid patients on a rice-diet regimen.

2. The high uptake of I^{131} is probably due to the iodine restriction in the diet.

3. This manifestation of restricted iodine intake does not develop until after three months on the diet.

4. No enlargement of the thyroid gland is associated with this phenomenon.

Acknowledgment

The authors would like to express their appreciation to Dr. Kempner and his associates for supplying patients and data.

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EFFECT OF METABOLIC INHIBITORS UPON IODIDE TRANSPORT IN SHEEP THYROID SLICES*†

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DURING incubation in saline media containing sufficient 1-methyl-2-mercapto-imidazole (Tapazole®) to inhibit organic iodinations, surviving thyroid slices accumulate inorganic iodide (1). An active transport has been implicated in this process and its physical optima have been defined (1). In the present experiments, the same preparation has been used to characterize further the biochemical phenomena which mediate the thyroidal transport of inorganic iodide. Thyroid slices have been suspended in serial dilutions of various metabolic inhibitors; the concurrent effects upon respiratory activity and the accumulation of iodide have been measured.

Recognizable difficulties are inherent in the use of metabolic inhibitors in cellular systems (2, 3). Most inhibitors have multifocal actions. Hence, activities in isolated enzyme preparations cannot be directly extrapolated to complex cellular phenomena. Furthermore, binding, degradation or volatilization of inhibitor may obscure quantitative relationships. Negative experimental results may merely reflect inadequate cellular penetration. These limitations are equally applicable to studies conducted with slices *in vitro* or with animals *in vivo*. The former technique, however, eliminates such variables as blood flow, concentration of inhibitor, sys-

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temic toxicity, and the indirect effects of extrathyroidal regulators (*e.g.*, pituitary).

METHODS

Experimental techniques have been presented in detail elsewhere (1). Fresh abattoir thyroid glands were secured from exsanguinated sheep or lambs (4) and carried to the laboratory in iced Dewar flasks. Glands were wrapped separately in cellophane; all tissues for a single experiment were derived from the same donor animal.

Krebs-Ringer-Phosphate (5) containing 1×10^{-3} M concentrations of Tapazole® and 0.7 to 1.0 microcurie per milliliter of carrier-free, inorganic I^{131} (KRP-Tap. 10^{-3}) was used as the suspending medium in all experiments. Inhibitors were added as the sodium salt during the preliminary preparation of the Krebs-Ringer solutions (KR). Sodium chloride within the KR was varied to maintain total osmolarity at 310 milliosmols per liter. KR solutions were adjusted to pH 7.4 with 0.3 N NaOH and HCl prior to the addition of radioactivity, Tapazole®, and phosphate buffer (pH 7.4) to yield KRP-Tap. 10^{-3}. Various dilutions of inhibitors were prepared by combining plain KRP-Tap. 10^{-3} with KRP 10^{-3} containing the appropriate inhibitor. Warburg vessels were kept on cracked ice and filled with 3.0 ml. of these mixtures.

Thin slices were sectioned from the central cores of thyroid lobes and immersed in chilled 0.15 M NaCl. Slices were briefly blotted on filter paper and amounts weighing 150–250 mg. were introduced into the main compartment of Warburg vessels; 0.2 ml. of 10 per cent KOH was added to the center well, and the flasks were incubated at 38°C. Following temperature equilibration and gassing with 100 per cent oxygen, oxygen consumption was measured by direct manometry (5) at ten-minute intervals for one hour. Slices were then quickly removed from the flasks, blotted, and weighed on the torsion balance. Since organic iodinations are completely inhibited in KRP-Tap. 10^{-3} (1), slices were digested directly in 2 N NaOH. All recovered radioactivity[1] was designated as glandular inorganic I^{131}. Suspending solutions were centrifuged, and residual I^{131} in the medium was determined quantitatively from portions of the supernatant liquid. Suitably diluted aliquots of the medium and of the glandular moieties were used for assay of radioactivity by means of a mica end-window Geiger-Mueller tube. Differential gradients for inorganic I^{131} between slice and suspending medium (S/M I^{131} ratios) were derived from the relationship:

$$\text{S/M } I^{131} = \frac{\text{counts/Gm. of tissue}}{\text{counts/ml. of medium}}$$

Cellular radioactivity was related to the final wet weight of slices. The initial wet weight of the slices was employed to estimate oxygen consumption (QO_2) for each flask (cu. mm. O_2/hour/mg.).

At least three separate experiments were performed with every inhibitor. All agents were investigated in multiple dilutions, to facilitate comparison with published studies of other systems. QO_2 and S/M I^{131} were related to values observed in control flasks containing KRP-Tap. 10^{-3} without inhibitor. Initial dilutions of inhibitor exceeding 1×10^{-2} M were not examined except in studies with L-histidine, dehydroacetic acid, and the monovalent anions.

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[1] Radioactive contamination due to faulty blotting could not elevate S/M ratios above 1.0.

TABLE 1. EFFECT OF METABOLIC INHIBITORS ON RESPIRATION AND IODIDE CONCENTRATION BY SHEEP THYROID SLICES*

Exper. No.	Inhibitor	Concentration (M)†	Control		Exper./Control (%)	
			QO_2	S/M I^{131}	QO_2	S/M I^{131}
		Inhibitors of Aerobic Processes				
42	Nitrogen	100%	0.50	42.1	4.0	3.1
132	Cyanide	1×10^{-2}	0.60	80.7	15.6	1.4
		5×10^{-4}			58.5	56.1
130	Azide	1×10^{-2}	0.36	60.3	32.0	1.6
		5×10^{-4}			88.4	69.5
209	Sulfide	1×10^{-2}	0.67	133.2	94.0‡	34.2
		1×10^{-3}			106.0	98.8
174	Arsenite	1×10^{-2}	0.32	42.9	15.6	21.9
		1×10^{-4}			78.1	82.3
159	Phloridzin	1×10^{-3}	0.50	110.4	90.0	67.2
125	Malonate	1×10^{-2}	0.32	32.6	106.2	96.0
159	Dehydroacetate	3×10^{-2}	0.50	110.4	84.0	55.9
		9×10^{-3}			92.0	86.6
210	*p*-Phenylenediamine	1×10^{-2}	0.36	27.1	102.8‡	4.1
		1×10^{-3}			94.4	57.6
133	Fluoroacetate	1×10^{-2}	0.33	29.5	39.3	46.8
		1×10^{-4}			84.7	74.1
		Sulfhydryl Inhibitors				
151	Mercuric	5×10^{-3}	0.65	146.4	9.2	6.4
		5×10^{-4}			76.9	25.3
		5×10^{-5}			84.6	62.8
82	Cupric	1×10^{-2}	0.51	66.6	74.5	17.6
		1×10^{-3}			86.3	77.9
205	Zinc	1×10^{-2}	0.34	32.5	52.9	31.1
		1×10^{-4}			97.1	87.1
134	Iodoacetate	1×10^{-2}	0.38	35.5	14.6	2.5
		1×10^{-4}			90.9	36.2
144	*p*-Chloromercuribenzoate	1×10^{-3}	0.32	29.3	31.3	7.8
		3×10^{-4}			60.0	11.6
		1×10^{-4}			93.8	79.5
216	Meralluride	2×10^{-3}	0.75	138.1	10.7	0.6
		1×10^{-4}			100.0	12.6
215	Mercaptomerin	2×10^{-3}	0.71	142.8	91.5	68.3
		5×10^{-4}			95.8	74.7
220	2,3-Dimercaptopropanol	1×10^{-3}	0.40	35.9	72.5	14.5
		1×10^{-4}			90.0	49.6
		Inhibitors of Carbonic Anhydrase				
142	Sulfanilamide	5×10^{-3}	0.40	24.1	110.0	105.0
195	Diamox	1×10^{-2}	0.56	72.9	91.1	88.9

* Representative experiments have been selected. Every inhibitor was tested in multiple dilutions and in at least three separate experiments.

† In the "phosphate-bond energy" section, concentrations of active agents have been selected which most clearly demonstrate dissociation of QO_2 and S/M I^{131}. For the other active inhibitors, the highest and lowest effective concentrations tested are presented. Only the highest concentration of inhibitors which had no effect is listed.

‡ For sulfide, 176.2 per cent; and for *p*-phenylenediamine, 227.8 per cent, prior to correction for autoxidation.

Exper. No.	Inhibitor	Concentration (M)†	Control		Exper./Control (%)	
			QO_2	S/M I^{131}	QO_2	S/M I^{131}
	Inhibitors of Alkaline Phosphatase					
147	L-Histidine	5×10^{-2}	0.24	12.6	104.1	111.9
147	L-Histidine-Tris§	5×10^{-2}	0.24	12.6	104.1	88.1
149	Beryllium	1×10^{-3}	0.44	42.3	93.2	90.3
149	Beryllium-Tris§	1×10^{-3}	0.43	34.3	109.3	105.2
	Inhibitors of Oxidative Phosphorylation					
145	2,4-Dinitrophenol	5×10^{-5}	0.35	50.8	48.6	2.2
		2.5×10^{-5}			117.1	53.3
		1.25×10^{-5}			162.9	91.1
		3.12×10^{-6}			128.6	109.8
154	2,4-Dinitro-6-phenyl phenol	5×10^{-5}	0.31	66.1	29.0	2.9
		5×10^{-6}			164.5	64.3
		5×10^{-7}			96.8	89.6
122	2,4-Dinitro-6-cyclohexyl phenol	5×10^{-5}	0.27	12.9	37.0	8.5
		7.5×10^{-6}			114.8	54.3
106	2,6-Dinitro-4-chlorophenol	5×10^{-5}	0.38	27.2	73.7	16.5
166	2,4-Dichlorophenol	1×10^{-4}	0.29	17.0	137.9	68.8
		2.5×10^{-5}			106.9	106.5
157	4-Nitrophenol	1×10^{-3}	0.30	19.2	30.0	5.7
		5×10^{-5}			120.0	80.7
204	2,4,6-Trinitrophenol	1×10^{-3}	0.33	39.9	187.9	10.8
		5×10^{-5}			106.1	61.4
158	2-Amino-4-nitrophenol	1×10^{-3}	0.32	20.3	112.5	46.8
		1×10^{-4}			106.3	97.5
203	2-Nitrophenol	1×10^{-3}	0.28	24.7	132.1	95.9
		1×10^{-4}			114.2	102.1
172	3-Nitrophenol	1×10^{-4}	0.38	42.2	107.9	94.8
165	Resorcinol	1×10^{-4}	0.36	31.8	97.2	93.1
101	2,4-Dinitrobenzoate	1×10^{-4}	0.29	15.3	106.9	86.3
	Anions and Group VII of the Periodic Table					
179	Iodide	5×10^{-2}	0.85	179.7	102.4	0.4
201		1×10^{-5}	0.28	17.1	103.6	93.6
179	Thiocyanate	5×10^{-2}	0.85	179.7	103.6	0.4
39		1×10^{-4}	0.52	83.9	109.6	26.6
179	Manganese	5×10^{-2}	0.85	179.7	31.3	1.6
160		5×10^{-3}	0.57	149.8	77.2	71.5
179	Bromide	5×10^{-2}	0.85	179.7	94.3	14.0
219		5×10^{-3}	0.26	19.3	115.4	88.6
179	Nitrate	5×10^{-2}	0.85	179.7	136.1	2.3
181		5×10^{-3}	0.48	31.4	93.8	23.9
179	Fluoride	5×10^{-2}	0.85	179.7	50.6	1.6
126		1×10^{-3}	0.34	43.6	105.9	98.6
196	Sulfate	1×10^{-2}	0.47	131.5	100.0	91.6
205	Perchlorate	5×10^{-2}	0.34	32.5	108.8	1.8
		1×10^{-3}			94.1	2.1

§ Inorganic phosphorus omitted from medium. Tris (hydroxymethyl) aminomethane, pH 7.4, used as buffer system.

RESULTS AND DISCUSSION

Reduction of general oxidative processes by anoxia or cold produces parallel inhibition of iodide accumulation (1). The effects are reversible and hence are presumably unrelated to structural or functional changes in components of the transport sequence. Obviously, inhibitor action cannot be evaluated without reference to the effects upon general cellular metabolism. As others have demonstrated (3, 6, 7), this correlation may be effected by the use of QO_2 as a nonspecific index of aerobic activity and S/M gradients as a specific measure of electrolyte transport.

Results with various classes of inhibitors are presented in Table 1. The categories were selected in order to examine circumscribed aspects of iodide transport. For each group, characteristic inhibitory patterns could be defined. The patterns were sufficiently distinct to suggest that the number of inhibitors within the separate categories could have been expanded with reasonable predictability.

In general, inhibitors produced three types of relationship (Fig. 1): a) simulation of the effects of anoxic anoxia, with parallel depression of QO_2

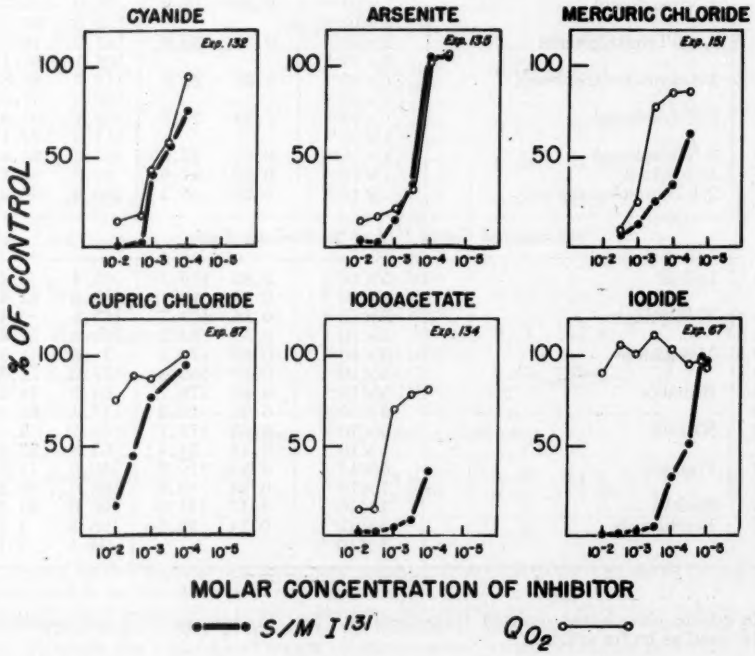

FIG. 1. Effect of metabolic inhibitors upon the respiration and concentration of iodide by sheep thyroid slices.

and S/M I^{131} at all effective concentrations (*e.g.*, cyanide, arsenite); b) greater inhibition of iodide transport than of QO_2 (*e.g.*, heavy metals); and c) depression of S/M gradients without change in oxygen consumption (*e.g.*, iodide).

1. *Aerobic processes*

Interference with aerobic processes by a variety of mechanisms diminished the accumulation of I^{131} in the slice system (Table 1).

Cyanide, azide and sulfide inhibit many enzyme systems dependent upon metalloproteins for their functional activity (*e.g.*, cytochrome oxidase, catalase, peroxidase, tyrosinase) (8). All three agents inhibited iodide transport in the present experiments. Their action may be ascribed to impairment of cellular respiration, since cyanide and azide depressed QO_2, and correction for autoxidation[2] accounted for the enhanced oxygen consumption observed in the presence of sulfide. P-phenylenediamine reduced S/M I^{131} gradients. This inhibition may also be attributed to interruption of aerobic processes since the oxidation of *p*-phenylenediamine by cytochrome c (9, 10) diverts cytochromal activities from normal electron transfer.

Phloridzin and sodium arsenite can impair the generation of adenosine triphosphate from inorganic phosphate and adenylic acid during the oxidation of succinate by washed tissue particles of mammalian kidney (11). Arsenite may also inactivate a variety of dehydrogenases (12, 13), whereas phloridzin at concentrations lower than those which inhibit glucose phosphorylation (14) interferes with the oxidation of pyruvic and citric acids (15, 16). Arsenite produced parallel inhibition of QO_2 and S/M I^{131}. Phloridzin (1×10^{-3} M) depressed iodide gradients to a greater extent than oxygen consumption.

Malonate (competitively) (17) and dehydroacetate (non-competitively) (18) inhibit the activity of succinic dehydrogenase. At high concentrations of dehydroacetate, enzymatic inhibition is associated with respiratory stimulation in minces of liver or kidney (18). Therefore, the observed dichotomy between QO_2 and S/M I^{131} at 3×10^{-2} M concentrations of dehydroacetate cannot be assigned much significance. Neither respiration nor iodide accumulation was significantly affected by malonate (1×10^{-2} M).

The enzymatic condensation of fluoroacetate with oxaloacetate yields a

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[2] This correction constitutes only a first-order approximation. Autoxidation is dependent upon pH and traces of heavy metals within the incubating medium (9). Since -SH groups in the suspended slices may react with traces of Cu^{++} in the KRP-Tap. 10^{-3}, autoxidation may be entirely different in the presence of tissue.

fluorotricarboxylic acid (probably fluorocitrate) (19). This product competitively inhibits the conversion of citrate to isocitrate by aconitase. In surviving thyroid tissue, 1×10^{-2} to 1×10^{-4} M concentrations of fluoroacetate produced parallel depression of iodide accumulation and respiration. A "halide" effect (due to liberation of anionic fluoride) may be excluded on the basis of the known stability of the CF bond (19).

Relative potencies of some of these agents were compared by incubating slices from a single gland with identical concentrations of the various inhibitors (Table 2).

From the foregoing data, it is apparent that any impairment of aerobic processes, whether by inhibition of cytochrome-cytochrome oxidase, by "poisoning" of aerobic dehydrogenases, or by competitive "jamming" of

TABLE 2. RELATIVE POTENCIES OF AGENTS WHICH INTERFERE WITH AEROBIC PROCESSES IN THYROID SLICES

(Exper. No. 175)

Inhibitor	Concentration	QO_2	S/M I^{131}
None		0.31	46.2
Partial anoxia	98% N_2, 2% O_2	0.12	25.8
Fluoracetate	1×10^{-2}M	0.10	19.1
Cyanide	1×10^{-2}M	0.08	1.3
Arsenite	1×10^{-2}M	0.06	9.2
Sulfide	1×10^{-2}M	1.02*	1.0
Azide	1×10^{-2}M	0.12	1.1

* Value not corrected for autoxidation.

the Krebs cycle, results in parallel reduction of QO_2 and S/M I^{131}. These effects resemble those of anoxic anoxia (1) and clearly implicate the tricarboxylic acid cycle as the major oxidative pathway of thyroidal metabolism. However, specific significance in iodide transport cannot be assigned to such ubiquitous reactions unless anionic exchanges are directly mediated by the cytochromal transfer of electrons (20).

2. *Sulfhydryl groups*

Functional integrity of –SH groups in the protein moiety is prerequisite for the activity of many hydrolytic enzymes, aerobic and anaerobic dehydrogenases, and enzymes mediating the transfer reactions of adenosinetriphosphate (21). In coenzyme A, the terminal sulfhydryl constitutes the reactive site for acyl-mercaptide linkage during the generation of high-energy thiol bonds (22). Disulfide linkage is present in lipothiamide, which acts as still another cofactor in the oxidation of pyruvate and α-ketoglu-

tarate (23). Obviously, the multiplicity of thiol-dependent reactions justifies only descriptive analysis of results obtained with substances which may inactivate sulfhydryl or disulfide groupings by oxidation-reduction (*cf.*, cupric salts), alkylation (*cf.*, iodoacetate), or mercaptide formation (*cf.*, *p*-chloromercuribenzoate) (42).

In the thyroid slice, all sulfhydryl reagents effected a dissociation of iodide transport and respiratory metabolism (Table 1, Fig. 1). At low concentrations of inhibitor, slice/medium gradients were uniformly depressed without significant change in oxygen consumption; much higher concentrations were required for comparable reductions of QO_2. On a molar basis, mercuric chloride was more effective than zinc or cupric salts, although quantitative intercomparisons may have been obscured by the precipitation of heavy metal phosphates.

Ionic iodide is evolved during the interaction of iodoacetate with –SH compounds to form alkylated derivatives; this I^{127} may have enhanced the apparent inhibition of transport by iodoacetate. Organic mercurials were slightly less potent than equimolar amounts of inorganic mercury. Merraluride sodium (Mercuhydrin®)[3] and mercaptomerin sodium (Thiomerin®)[4] were examined as structural representatives of the organic mercurial diuretics. Mercuhydrin® contains the conventional bond between the second valence of mercury and the secondary nitrogen of theophylline; theophylline is displaced as linkage to –SH groups occurs. Contrariwise, in Thiomerin®, a monothiol has been substituted for the theophylline residue and it has been postulated that combination with –SH moieties is effected by exchange reactions (25). Whether the diminished potency of Thiomerin® in the thyroid-slice system results from the intrinsic thiol grouping cannot be assessed. Conceivably, this configuration might also impair the formation of stable cyclic mercaptides with 2,3-dimercapto-1-propanol (BAL).[5] Simultaneous incubation of thyroid tissue with 5×10^{-4} M concentrations of 2,3-dimercapto-1-propanol and meralluride sodium afforded complete protection against the organic mercurial[6]; similar amounts of the dithiol did not significantly modify the action of mercaptomerin sodium (Table 3). Thyroidal oxygen consumption was depressed

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[3] N (β-hydroxymercuri-γ-methoxy)-propyl-N′-succinylurea: Lakeside Laboratories' Inc.

[4] N-(γ-carboxymethylmercaptomercuri-β-methoxy)propylcamphoramic acid disodium: Wyeth Laboratories.

[5] (2,3-Dimercapto-1-propanol) 10%; benzyl benzoate 20%; in peanut oil: Hynson, Wescott & Dunning, Inc.

[6] The reversal of the individual inhibitory effects of Mercuhydrin and BAL by adding them simultaneously would suggest that the theophylline fraction of the former and the suspending solvents of the latter are functionally inert at this concentration.

by 2,3-dimercapto-1-propanol alone, as has been reported with slices of brain, kidney and diaphragm (26). Barron has suggested that the direct effect of 2,3-dimercapto-1-propanol (BAL) upon oxidative metabolism may result from combination with heavy metal prosthetic groups, or from delay in the oxidation of cytochrome c by cytochrome oxidase, or from inhibition of –SH enzymes by the oxidized dithiol (26). In the present experiments, the latter possibility seems most likely, since BAL reduced S/M I^{131} to a greater extent than QO_2.

The effects of sulfhydryl reagents are not without parallel in other systems (3), and hence may reflect properties of active transport in general, rather than iodide transport in particular.

TABLE 3. THE EFFECTS OF ORGANIC MERCURIALS AND 2,3-DIMERCAPTOPROPANOL UPON SHEEP THYROID SLICES

(Exper. No. 222)

	Concentration ($\times 10^{-4}$)	Flask contents*					
2,3-Dimercaptopropanol	2.5	−	+	−	−	+	+
Mercaptomerin sodium	2.5	−	−	+	−	+	−
Meralluride sodium	2.5	−	−	−	+	−	+
	QO_2	0.33	0.29	0.29	0.07	0.29	0.36
	S/M I^{131}	45.2	10.5	31.7	0.8	33.3	47.9

* The plus sign indicates the presence of inhibitor in the flask.

3. *Carbonic anhydrase*

Transcellular ionic exchanges in many systems are dependent upon the rate of hydration of carbon dioxide to yield H^+ and HCO_3^- (27–30). Carbonic anhydrase catalyzes this reaction. Convincing demonstration of intrathyroidal carbonic anhydrase is not available. Nonetheless, since maintenance of electrical neutrality might require the concomitant entrance of cation or egress of negatively charged intracellular particles during net accumulation of iodide (*e.g.*, when carrier I^{127} is varied), inhibitors of carbonic anhydrase were studied. Sulfanilamide and 2-acetyl-amino-1,3,4,-thiadiazole-5-sulfonamide[7] halve the activity of erythrocyte carbonic anhydrase at concentrations of 6×10^{-6} M and 1×10^{-8} respectively (31). Sulfanilamide (5×10^{-3} M) did not significantly alter QO_2 or S/M I^{131}. The 10 per cent depression of iodide transport occasionally seen with 1×10^{-2} M

[7] 2-Acetyl-amino-1,3,4-thiadiazole-5-sulfonamide: Lederle Laboratories Division, American Cyanamid Co.

concentrations of 2-acetyl-amino-1,3,4-thiadiazole-5-sulfonamide is of questionable significance.

These results must be interpreted with caution, and do not preclude the possibility or H^+ of HCO_3^--exchange during iodide transport. Carbonic anhydrase occurs in great excess in most tissues in which it has been demonstrated. In the stomach, 99.5 per cent of the enzyme must be inhibited to achieve 50 per cent reduction in the interaction between H_2O and CO_2 (32). Moreover, Davenport has suggested that enzymic catalysis may be unnecessary, "if the rate of reaction required by the metabolic activity of the tissue is not limited by blood flow or other mechanical factors" (32). Thus, the present data indicate only that gradients for iodide *in vitro* are not limited by amounts of Diamox® or sulfanilamide which may impair the enzymatic formation of carbonic acid in other systems.

4. *Alkaline phosphatase*

Dempsey and Singer have reported histochemical demonstration of alkaline phosphatase activity within the thyroid gland (33). Slices of sheep thyroid tissue were incubated both with and without inorganic phosphorus in media containing L-histidine or beryllium (as the hydroxide or sulfate). In concentrations greater than are required for the inhibition of alkaline phosphatases in intestine, bone and kidney (34–37), these agents did not modify S/M I^{131}.

5. *Phosphate-bond energy*

The establishment of concentration differentials for iodide entails selective transfer across cell membranes and continued concentration of accumulated ion. All the integrated movements are directed against a chemical potential gradient. Obviously, free energy must be expended at some phase of the transport sequence. To determine the source of this energy, experiments were conducted with a series of substituted benzene compounds, the effects of which upon the aerobic esterification of inorganic phosphate have been characterized in washed tissue particles of mammalian kidney (cyclophorase system) (11). In the cyclophorase system, the generation of adenylpyrophosphates can be dissociated from oxidative processes by 2,4-dinitrophenol (11, 38) and related nitrophenols of high structural specificity in concentrations of 2.5×10^{-4} M (11). The nature of the substrate and the age of the donor animal can influence the augmented respiration which may accompany this "uncoupling" of oxidation and phosphorylation (11, 39).

In the thyroid-slice preparation, titration with some of the substituted phenols produced a characteristic inhibitory pattern (Fig. 2). Agents which depress P/O ratios in the cyclophorase system produced respiratory sup-

pression and virtual abolition of iodide transport in concentrations of 5×10^{-5} M (Table 1). Within a limited range of greater dilution, S/M I^{131} remained partly depressed despite elevation of oxygen consumption above control levels. At peak respiratory stimulation (*i.e.*, 40–60 per cent) S/M ratios approached control values. The relationships simulate those of the reported inhibition of cell cleavage and phosphorylation in fertilized arbacia eggs by concentrations of dinitrophenol greater than those which produce maximal augmentation of oxygen consumption (40, 41). Save for 4-nitrophenol, 2,4-dichlorophenol, and 2,4,6-trinitrophenol (picric acid),

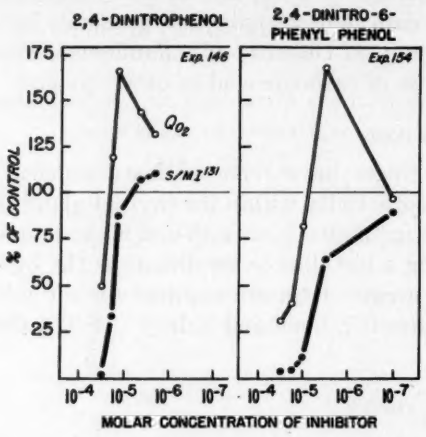

FIG. 2. Dissociation of iodide transport and oxygen consumption by agents which uncouple oxidative phosphorylations.

agents which are inert in the cyclophorase system exerted no significant effect upon QO_2 and S/M I^{131} when tested in concentrations of 1×10^{-4} to 2.5×10^{-5} M. The dihalophenols and 4-nitrophenol uncouple oxidative phosphorylations in the arbacia preparation (41, 42). It would seem reasonable to attribute their efficacy in the thyroid slice to a similar mechanism.

Trinitrophenol did not conform to the general pattern observed with the potent substituted phenols (Fig. 3). In the presence of 10^{-3} M concentrations of picric acid, oxygen consumption was elevated and iodide transport was reduced. However, when picric acid was further diluted (1×10^{-4} M to 2.5×10^{-5} M), QO_2 returned to control values although depression of S/M I^{131} persisted. In surviving kidney tissue, trinitrophenol (5×10^{-5} M) similarly inhibits the anionic accumulation of phenol red and *p*-aminohippurate without altering respiratory metabolism (6, 7).

The structural specificity of "uncoupling agents" was examined by

incubating thyroid slices with higher concentrations of some of the "inert" phenol derivatives (Fig. 3). 2-Nitrophenol enhanced QO_2 slightly but did not affect gradients for iodide in concentrations of 1×10^{-3} M. Oxygen consumption was profoundly increased and iodide transport was depressed in the presence of 2-amino-4-nitro-phenol in concentrations of 1×10^{-3} M. The results mirrored the activities of a hundredfold to a thousandfold greater dilutions of 2-4-dinitrophenol and allied derivatives. These observations demonstrate the usefulness of concentration curves for characterizing

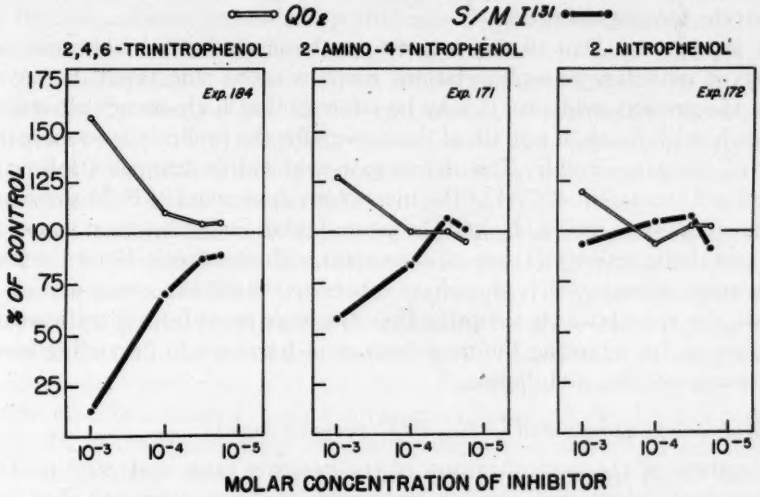

FIG. 3. The effects of substituted phenols on the concentration of iodide by thyroid slices. For discussion, see text.

metabolic inhibitors, and suggest that many of the substituted phenols may differ only in relative potencies.

Some of these compounds have been tested by others. Weiss noted that 1×10^{-3} M concentrations of 2,4-dinitrophenol and 4-nitrophenol diminished the formation of radioactive "diiodotyrosine" and "thyroxine" from NaI^{131} in unblocked slices of beef thyroid tissue (43). On the assumption that 4-nitrophenol does not affect oxidative phosphorylations, Weiss attributed the action of the substituted phenols to competitive inhibition, and combined this evidence with other data[8] to suggest that "phosphate

[8] Weiss observed that inorganic P^{32} was not esterified during incubation with cell-free thyroidal homogenates and that 1×10^{-3} M concentrations of 2,4-dinitrophenol did not impair the organification of carrier-free I^{131} by this system (43). Fawcett and Kirkwood have recently presented evidence that organic iodinations do not proceed beyond the enzymatic formation of free monoiodotyrosine in Weiss' homogenates (44).

bond energy is not directly coupled to the biosynthesis of thyroxine." Weiss' interpretations warrant modification in the light of the present data.

First, 4-nitrophenol cannot be classified as an inert competitive inhibitor. Secondly, as has been stated previously (1), organic iodinations cannot be evaluated in cellular systems unless reference is made to the availability of ionic iodide. In sheep thyroid slices, 1×10^{-3} M concentrations of 4-nitrophenol and 2,4-dinitrophenol diminish QO_2 and reduce S/M gradients to 1.0 or less. Consequently, even in the presence of intact mechanisms for hormone manufacture, such low gradients would provide little anionic substrate for organification.

It is apparent that in the organic synthesis of thyroid hormone, the status of oxidative phosphorylations remains to be elucidated. However, from the present evidence, it may be inferred that high-energy phosphate bonds furnish much, if not all, of the energy for the preliminary concentration of inorganic iodide. The deterioration of iodide transport following prolonged storage at 4°C (1),[9] the inconstant depression of S/M gradients in phosphate-free media (1), and the general relationship between transport and metabolic activity (1) are all consonant with this thesis. Since the local generation of energy-rich phosphate esters may limit the accumulation of iodide, the speculation is tempting that this may provide an intrathyroidal mechanism for adapting the manufacture of hormone to prevailing levels of aerobic cellular metabolism.[10]

6. *Monovalent anions and Group VII (periodic table)*

Members of the seventh group of the periodic table and certain other monovalent anions were studied because it has been suggested that they may compete for the mechanism of iodide transport (47, 48) (Table 1). Concentrations of carrier I^{127} exceeding 1×1^{-5} to 2×10^{-5} M produced progressive diminution of S/M gradients, although the calculated content

[9] Aging may diminish the phosphorylation ability of washed tissue particles without comparably affecting their oxidative capacity (45, 46).

[10] Since the completion of this manuscript, VanderLaan and Caplan, and Halmi and Spirtos have reported a reciprocal relationship between thyroid/serum gradients for inorganic iodide and either total thyroidal iodine (55) or some more specific intrathyroidal iodinated organic compound, the concentration of which would "parallel that of total thyroidal iodine, and which could be thyroid hormone proper" (56). The foregoing data suggest that such an intrathyroidal regulator might act by limiting the generation of adenylpyrophosphates within the thyroid. Lardy has proposed that thyroid hormone may delimit the efficiency with which phosphorylations are coupled to certain oxidations (57). If such is indeed the case, it is conceivable that intrathyroidal stores of hormone could modify further synthesis within the parent gland by regulating the availability of energy for the accumulation of hormonal precursors. Experiments are in progress to test this hypothesis.

of stable iodide within the slices was augmented (1). Oxygen consumption was not modified in media containing as much as 5×10^{-2} M concentrations of I^{127}. Manganese (5×10^{-2} M to 10^{-3} M) depressed both S/M I^{131} and QO_2. Bromide[11,12] (5×10^{-2} M) partially inhibited S/M gradients without depressing oxygen consumption. Thiocyanate, like I^{127}, virtually abolished the accumulation of I^{131} (S/M ratios of 0.7 to 1.0) in concentrations of 5×10^{-2} M. Lack of respiratory depression would minimize the possibility that thiocyanate acts within the thyroid by local degradation to cyanide (49). S/M gradients were partly depressed by concentrations of fluoride of 3×10^{-3} M; concentrations of 5×10^{-2} were required to diminish QO_2 significantly. Since fluoride exerts multiple metabolic actions in both glycolytic (50) and oxidative systems (3, 6), it need not act solely by substitution for iodide. The effects of perchlorate and nitrate,[12,13] paralleled their relative potencies *in vivo* (48, 51) and were unassociated with depression of respiratory metabolism. Work is in progress to define the minimum effective concentrations of nitrate and perchlorate.

Certain similarities are striking when the effects of these anions are compared with their activities in other anionic systems of transport. For example, the accumulation of *p*-aminohippurate (PAH) by kidney slices may also be impaired by substituting other anions for chloride in the incubating medium (52). Larger concentrations are required than in the present experiments; nonetheless, the inhibitory potential of anions in the PAH system may be arranged in the following sequence: $SCN>I>NO_3>Br>SO_4>PO_4$. None significantly diminishes respiratory activity. Fluoride has not been included, since it depresses the QO_2 of renal tissue (3, 7). In kidney (7), and in thyroid slices, the reduction of oxygen consumption by fluoride is not nearly as great as the inhibition of anionic transport. The significance of these parallelisms is not clear. Competitive transport in the traditional sense seems unlikely. Published reports do not favor concentration of thiocyanate by thyroid tissue (53); S/M gradients for thiocyanate cannot be demonstrated by suspending kidney slices in 1×10^{-3} M concentrations of thiocyanate (52). However, since the similarities in diverse anionic systems appear to be more than merely fortuitous, the possibility cannot be excluded that many anions share some single stable or unstable component during transport.

It is of interest in this connection that cationic transport (*e.g.*, the ac-

[11] Sodium Bromide Merck, Reagent Grade, "Maximum impurities: Iodide (I) 0.00%"—Merck & Co., Inc.

[12] Contamination by ionic iodide is insufficient to account for the effects observed *in vitro*.

[13] Sodium Nitrate Merck Crystals, Reagent Grade, "Maximum impurities: Iodate (IO_3) 0.0005%"—Merck & Co., Inc.

cumulation of potassium by kidney slices) is unaffected by media containing bromide, nitrate (54), or thiocyanate (3) as the major anion.

SUMMARY AND CONCLUSIONS

Slices of sheep thyroid glands have been incubated in solutions containing serial dilutions of various metabolic inhibitors in addition to concentrations of 1-methyl-2-mercaptoimidazole sufficient to eliminate organic iodinations. Inhibitory patterns have been defined by measuring the accumulation of inorganic I^{131} and the cellular consumption of oxygen. Although the available data do not justify an integrated formulation of the mechanism for thyroidal transport of iodide, certain preliminary conclusions may be derived.

Establishment of concentration differentials for iodide requires energy which is generated by cellular aerobic oxidations and which is channeled through high-energy phosphate bonds. Thus, thyroidal iodide transport can be interrupted by inhibitors which interfere with aerobic metabolism, or which dissociate phosphorylation from oxidation. Sulfhydryl reagents and certain monovalent anions appear to affect other components of the transport system. Specific inhibitors of carbonic anhydrase and alkaline phosphatase do not limit the accumulation of inorganic iodide in thyroid slices.

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CONVERSION OF THYROXINE TO 3-5-3′-TRIIODOTHYRONINE *IN VIVO**

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THE principal iodinated compound of the blood is thyroxine (1, 2, 3). In addition, small amounts of triiodothyronine have been found in the blood of clinically euthyroid subjects and of patients with hyperthyroidism (4). Both thyroxine and triiodothyronine have been identified in the normal rat and ox thyroid gland (5). However, it has not been shown whether the triiodothyronine which is present in the peripheral blood is secreted directly by the thyroid gland, or whether it is derived from degradation of previously secreted thyroxine.

The present study demonstrates that triiodothyronine can be formed in the human subject from thyroxine in the absence of the thyroid gland, and confirms in man the finding of Gross and Leblond (6) that "unknown 1" (later shown to be triiodothyronine) can be formed in certain peripheral tissues in the thyroidectomized animal.

METHOD

Six patients with typical athyreotic myxedema were chosen. The diagnosis in each case was proved by measurements of basal metabolic rate and of serum concentration of protein-bound iodine (0.0 to 1.3 μg. per 100 ml.). Five patients were then given thyroxine in doses varying from 50 to 300 micrograms daily. By analysis in a Kieselguhr column it was shown that the preparation was at least 95 per cent thyroxine, the remaining iodine being present as iodide; no triiodothyronine could be detected. The thyroxine was administered orally to 1 of the patients and subcutaneously to the other 4. The medication was continued for two to four weeks until the clinically euthyroid state was nearly or entirely reached. The sixth patient received no thyroxine. At this juncture each patient received intravenously approximately 500 microcuries of I^{131}-labeled thyroxine.[1] All samples of I^{131}-labeled thyroxine contained a small amount of radioactive material with the same Rf as triiodothyronine in butanol-ammonia and butanol-dioxane-am-

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[1] The labeled thyroxine was obtained from Abbott Laboratories, Various lots assayed from 10 to 20 millicuries per milligram.

monia (no more than 2.2 per cent of the total radioactivity in the thyroxine fraction).

Serial blood samples were collected at intervals ranging from five minutes to 114 hours after administration of the labeled thyroxine. A 3-ml. portion of serum was extracted at pH 2-3 with butanol, evaporated, and chromatographed one-dimensionally in butanol-ammonia, or butanol-dioxane-ammonia, and in some cases two-dimensionally in butanol-acetic acid and butanol-dioxane-ammonia (7). Thyroxine and triiodothyronine spots were identified by adding carriers and spraying with diazotized sulfanilic acid (7). The spots were cut out and measured directly in a well-crystal scintillation detector. The background count was taken on various parts of the chromatogram between the triiodothyronine spot and the solvent front, and was always found to be considerably greater than the detector background count.

The samples of serum from 4 of the patients were measured for content of total labeled iodine. Of the total labeled iodine, 88 per cent or more was precipitable with trichloracetic acid.

RESULTS

The results of the chromatographic analyses are shown in Table 1. In the two experiments in which early measurements were made, new triiodothyronine appeared in the serum within three hours. The proportion of triiodothyronine reached its maximum value between three and twenty-eight hours, and subsequently declined. The maximum values for labeled iodine in the triiodothyronine band were between 4 and 7 per cent of that in the thyroxine band. After seventy-two to ninety-six hours the radioactivity in the triiodothyronine spot had returned to its initial value.

TABLE 1. PERCENTAGE OF THYROXINE PLUS TRIIODOTHYRONINE PRESENT AS TRIIODOTHYRONINE

Time after labeled thyroxine	Patient No.					
	1	2	3	4	5	6
0	1.0	1.0	1.0			1.4
5 min.				1.9	2.2	
30 min.				1.8		
1 hr.				2.1	2.1	
3 hrs.				4.5	2.6	
6 hrs.				5.0	5.0	3.2
12 hrs.						4.0
18 hrs.	5.7		6.7			
24 hrs.	4.8		5.6	3.4	3.1	2.2
28 hrs.		5.8				
32 hrs.				2.5		1.7
48 hrs.	3.0	3.8	4.0	2.4	2.2	1.5
68 hrs.	1.4		3.3			
72 hrs.		2.0				1.5
96 hrs.						1.3
114 hrs.			1.2			

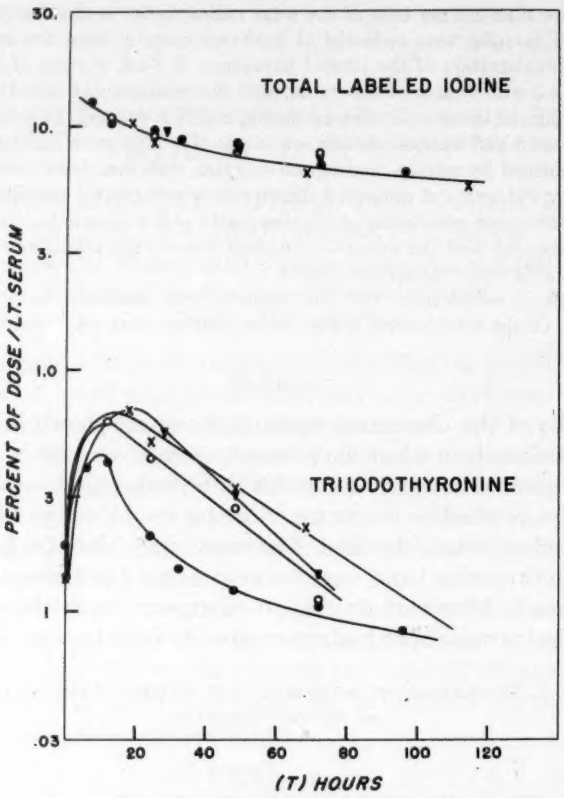

FIG. 1. The disappearance of labeled iodine and the appearance of triiodothyronine in the blood of 4 patients after administration of I^{131}-labeled thyroxine. The ordinate is the percentage of the administered dose per liter of serum, and the abscissa is the time in hours.

The serum concentrations of labeled iodine and of triiodothyronine are shown in Figure 1. The upper curve is the total serum concentration of labeled iodine. The extrapolated concentration at zero time was 13.9 per cent of the administered dose per liter of serum. The lower curves are calculated from the ratio of labeled iodine in the triiodothyronine band to that in the thyroxine band. The zero points on the triiodothyronine curves are the contaminating amounts of substance in the injected solution which had the chromatographic properties of triiodothyronine. Specific-activity curves could not be calculated because the absolute amounts of triiodothyronine and thyroxine were not known, but the relative slopes of the curves for total labeled iodine and for triiodothyronine would not be changed by this calculation, providing these concentrations were constant.

The unexpected finding of interest was the steeper slope of the triiodothyronine curves as compared to the curve of total labeled iodine.

COMMENT

Hypothyroid patients were chosen for this study. All but 1 were rendered nearly or completely euthyroid, in order that any possible remaining functioning thyroid tissue would be completely depressed. Further, the patients were treated with pure thyroxine rather than desiccated thyroid, in order to preclude the possibility of exchange between I^{131} from the labelled thyroxine and I^{127} from triiodothyronine in desiccated thyroid.

The rise in serum concentration of labeled triiodothyronine cannot be attributed to simple exchange. No stable triiodothyronine was given to these patients. The increase in labeled triiodothyronine in the serum of the patient who received no stable thyroxine was entirely similar to that in the other patients; this suggests that the administered labeled thyroxine did not exchange with triiodothyronine previously formed *in vivo* from the administered thyroxine.

It may be seen from Table 1 that the amounts of triiodothyronine found in the serum of athyreotic patients are small, and it may well be that considerably greater amounts could have been found in other organs or tissues. In their study on thyroidectomized mice, Gross and Leblond (6) were unable to detect "unknown 1" (triiodothyronine) in the sera of the animals by autoradiographic methods, although they found detectable amounts in other tissues.

It appears, therefore, that the thyroid gland is not essential for the formation of triiodothyronine, and that some other organ is able to perform the partial deiodination of thyroxine. In this connection the recent experiments of Albright *et al.* (9) are worthy of comment. These authors have reported that kidney slices *in vitro* are able to effect this conversion. Roche *et al.* (10), however, were unable to find triiodothyronine in the rat after injection of labeled thyroxine.

The concentration of labeled iodine in the serum of these patients was remarkably uniform. It is not clear why the slope of the thyroxine decay curve was distinctly less than the variable slopes of the triiodothyronine decay curves. Although the finding does not suggest a simple precursor-product relationship, fluctuations in the serum concentrations of stable thyroxine and triiodothyronine may have occurred and obscured the expected relationship between the slopes of the curves.

SUMMARY

Small amounts of triiodothyronine have been detected in the blood of 6 athyreotic humans after administration of I^{131}-labeled thyroxine.

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Review

ALDOSTERONE—A REVIEW*

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IN 1934 an amorphous fraction of adrenal extracts was recognized to have a particularly high life-maintaining activity. Oddly enough, although knowledge of other adrenal hormones accumulated in profusion, it took two decades before a specific crystalline component of this fraction, aldosterone (electrocortin), was isolated and characterized. Crystalline aldosterone has been available for less than two years and only in the amount of a few milligrams. Nevertheless, remarkable progress has been made in characterizing the biologic activities and probable physiologic role of the hormone. This is largely due to the fact that a wide background of knowledge and experience concerning other adrenal steroids has been of great assistance in such studies.

Historical sequences. Three lines of investigation have led to the current picture, tentative though it is, of aldosterone and its significance. The first of these lasted approximately two decades (1934–1953) and concerned a study of the nature of the amorphous principle in adrenal tissue and adrenal effluents. Led in its final stages by Simpson and Tait, this study culminated in the chemical characterization of aldosterone by these authors and by Reichstein, Wettstein and their collaborators. The work along these lines prior to 1950 has been briefly reviewed by Ingle (43).

Another influential development was the synthesis of desoxycorticosterone by Steiger and Reichstein in 1937. This was followed by a deluge of biologic studies, most of which were made with the hypothesis in mind that desoxycorticosterone closely resembled a natural electrolyte-regulating adrenal hormone.

The third line of the study, opened by Luetscher and his colleagues in 1950, concerned the existence of a urinary sodium-retaining substance, particularly high titers of which were present in diseases characterized by

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edema. Their work led to the identification of the urinary substance as aldosterone.

For reasons of convenience rather than of chronology, desoxycorticosterone will be the first of these lines of study considered.

Desoxycorticosterone. In 1937 Steiger and Reichstein (108) synthesized 11-desoxycorticosterone.[1] Later Reichstein and von Euw (89) found small amounts in adrenocortical extracts, as did Zaffaroni and Burton (126) and Mattox (75).

The role of DC in the history of the adrenal field has been an influential but enigmatic one. For a long time it was the only adrenal steroid available in quantity by synthesis. It exerted profound effects as substitution therapy in adrenal insufficiency, particularly because of its influence on electrolyte excretion, and was of great value in the treatment of Addison's disease. It was soon evident, however, that DC did not provide complete substitution for the natural adrenal secretions. It was generally regarded as being the probable synthetic prototype of an electrolyte-regulating adrenal hormone, the natural form of which was present in the "amorphous fraction"—now presumably aldosterone. As indicated subsequently, it is apparent that although the actions of aldosterone do indeed resemble in part those of desoxycorticosterone, they are far from identical.

Is desoxycorticosterone a hormone? Hechter (42) reported that it was present in small quantity in the perfusates of adrenal glands *in vitro*. The work of Wettstein *et al.* (124) suggests the possibility that DC may be a natural precursor of aldosterone. Farrell and co-workers (25) have recently advanced the subject greatly with the demonstration that DC is released into the adrenal vein blood of dogs, and that the amounts produced are reduced by hypophysectomy and increased by administration of ACTH. They concluded, however, that the blood titers were not high enough for the substance to exert any significant biologic action. Possibly a small amount of DC escapes into the blood stream in the process of biosynthesis of other corticoids.

THE AMORPHOUS FRACTION

Significant chemical work on the hormones of the adrenal cortex dates from 1930, when Swingle and Pfiffner (113) and Hartman and Brownell (40) prepared the first adrenal extracts recognized to serve as substitution therapies for indefinite periods in the absence of endogenous adrenal function.

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[1] This substance, also known as cortexone, is generally used as its acetate and in this paper will be designated as DCA—or DC for the free alcohol. The abbreviation SRF will be used for the aldosterone-like sodium-retaining factor of urine which in some, but not all instances has been identified as aldosterone.

In 1934 Wintersteiner, Vars and Pfiffner (125, 85), having removed various crystalline substances from adrenal extracts, had a noncrystalline residue which assayed at 200 to 400 dog units per milligram. This residue was much more active than the separated crystalline components. Kendall, Mason and associates (51) made similar preparations, which were more active than desoxycorticosterone. In the Upjohn Laboratories, related studies by Cartland, Kuizenga and associates (53, 9) showed high activity in an amorphous fraction, which again could not be attributed to desoxycorticosterone or other known corticoids.

Hartman and colleagues (115, 41) studied a "sodium-retaining factor" from adrenal extracts and concluded on both chemical and biologic grounds that it differed from other known corticoids. Curiously, the Hartman sodium-retaining extracts, unlike the amorphous factors of other investigators, were not effective in life-maintenance—at least in cats. Doses of more than 1.5 mg. did not cause eosinopenia in mice (DC was active in doses of 150 μg.) (94) and in that respect the extract differed from crystalline aldosterone (30, 107).

Most of the biologic work with the early "amorphous fractions" was carried out with the preparations of Kendall, Mason and associates. Aside from their own work, they made these extracts available to other investigators, thus permitting them to be fairly extensively characterized.

Their "amorphous fraction" contained about half of the life-maintaining activity found in whole adrenal extracts and would maintain adrenalectomized dogs in doses of 1–2 μg./Kg./day (74). This fraction did not inhibit growth or cause thymic and adrenal atrophy as effectively as did corticosterone (121), and its actions on carbohydrate metabolism were weak (Wells and Kendall (122)). It was found, however, to provide some lactation-supporting action (29), and protection against water intoxication (24) and the stress of cold (50). It would sustain weight gains at a dosage level of 5 μg. per day in adrenalectomized rats and also maintain muscular work capacity to some degree (Ingle and Kendall (44)). The significance of the latter observation was enhanced by the subsequent finding that, in tests of muscle work, whole cortical extracts were more effective than any known crystalline corticoids (45).

Although the preparations used were not pure, the cumulative implication of these findings was that the "amorphous fraction" had a wider range of biologic activity than DC, and in certain respects a higher potency than any known corticoid.

In 1952 Simpson, Tait and co-workers began to publish the results of a systematic re-investigation of the activity in the amorphous fraction of adrenal extract. They made skillful use of two new tools. The first was a bioassay method (96), highly sensitive for the detection of "mineralo-

corticoid" activity.[2] The second was the application of new methods of chromatography, particularly those of Zaffaroni (6) and of Bush (7), to the isolation and identification of the substance causing this activity.

Using these methods, they showed again that the potency of adrenal extracts in affecting sodium and potassium excretion was greatly in excess of what could be accounted for by the content of any known adrenal steroid or combination of adrenal steroids (114, 38, 37). They showed further that the mineralocorticoid activity similar to that in adrenal extracts was also present in adrenal venous blood (99), thus adding great strength to the assumption that a true hormone was involved.

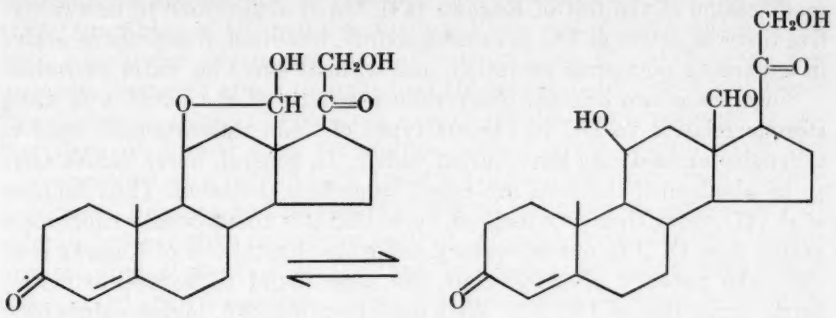
IDENTIFICATION OF ALDOSTERONE

A productive collaboration was then begun between the teams of Simpson and Tait, working at the Courtauld Institute, Middlesex Hospital, University of London; Wettstein and Neher, Ciba, Ltd., Basle; and von Euw and Reichstein, University of Basle. This program led to the isolation and crystallization of aldosterone, the announcement of which was published in 1953 (100). Shortly after this Mattox, Mason and Albert (76) also crystallized aldosterone. In the meantime, noncrystalline preparations from adrenal sources were obtained by Haines *et al.* (52) and by Kendall (cited by Swingle (110)), which were of the same order of potency as aldosterone. Simultaneously various workers were purifying from urine a substance which later proved to be aldosterone (67).

There was intense interest in the chemical nature of electrocortin for several months after its crystallization. In steroid centers, much laboratory as well as speculative effort was devoted to the problem of its constitution. Some of the hypothetical efforts achieved print but not verity. The problem was solved by the same team which first crystallized the hormone: in 1954 Simpson, Tait, Wettstein, Neher, von Euw, Schindler and Reichstein announced that electrocortin was 11β-21-dihydroxy-3,20-

[2] The recent development of the whole mineralocorticoid problem has depended upon sensitive methods of detection of mineralocorticoid activity in adrenal extracts and body fluids. The methods most used, aside from those of Simpson and Tait, are those of Johnson (46), Kagawa, Shipley and Meyer (48), Marcus, Romanoff and Pincus (72), and Singer and Venning (105). Sensitivity is increased if test substances are injected intravenously (57). Most laboratories which have used these methods have developed their own modifications of them. Two potential sources of error in such determinations should not be overlooked: a) if a urine, tissue extract or solvent (65) is toxic, it could cause a reduced glomerular filtration rate and in an acute test give the false appearance of causing a steroid-like sodium retention; b) if the test fluid contains much cortisone or hydrocortisone, these substances may cause increased sodium excretion and thus vitiate an assay.

diketo-4-pregnene-18-al, or the 18-aldehyde of corticosterone (101–103). They proposed the name *aldosterone*. This structure was unique among the hormonal steroids in two respects, 1) the presence of an 18-aldehyde group, and 2) the fact that in solution it existed largely in a second form, the 11-hemiacetal. Confirmation of this structure was provided by Sarrett *et al.*



Aldosterone

(39). Wettstein (123) has recently reviewed the chemical background of the whole problem.

Biologic work with aldosterone has been impeded by the fact that only trivial amounts (45–95 μg./Kg. in beef adrenals) exist in adrenal extracts (123) and these can be isolated in pure form only at great expense. Wettstein, Kahnt and Neher (124) found that the yield from beef adrenal brei could be enhanced several fold by aerobic incubation with substrates of the citric-acid cycle, such as nicotinamide, adenosine triphosphate (ATP), or ascorbic acid. This was not true of hog adrenal brei, which normally contains some twelve times more aldosterone than beef. The addition of desoxycorticosterone to the incubated beef adrenal brei resulted in a considerable further increase of the aldosterone content. The recent work of Luetscher, Neher and Wettstein (67) indicates that the amounts of aldosterone present in the urine of nephrotic patients may be large enough to make certain types of human urine the best natural source. Aldosterone will not, however, be available in quantity until an economic method of synthesis is discovered.

BIOLOGIC PROPERTIES OF CRYSTALLINE ALDOSTERONE

Estimates of activity by means of electrolyte excretion

The first work with crystalline aldosterone concerned its effects on sodium and potassium excretion. These observations served as a basis

for standardization procedures. Simpson and Tait found in their test, based on the ratio of Na^{24} to K^{42} excretion in adrenalectomized rats, that aldosterone had one hundred and twenty times the potency of desoxycorticosterone (107). The minimal effective dose of aldosterone approximated 0.01 μg. per rat. Desaulles, Tripod and Schuler (23), using a modification of the test of Kagawa (48), found aldosterone to be twenty-five times as active as DC in causing sodium retention, five times as active in enhancing potassium excretion, and without effect on water excretion.

Since these two original observations, reports of the potency of aldosterone relative to DC in various types of "mineralocorticoid" tests in adrenalectomized rats have varied widely. In general, lower values seem to be obtained if the tests are based on sodium retention. Thus Johnson *et al.* (47), using their own method, agree that it is about twenty times more active than DC.[3] In our laboratory, using the original test of Kagawa *et al.* (48), the potency of aldosterone has been found to be approximately thirty times that of DC (11). With one exception (90), higher values have been reported in assays based on urinary Na/K ratios, *viz.*, eighty-five to a hundred times DC (76, 77); forty-eight times DC (26); and several hundred times DC (39). Presumably these differences are due largely to variations in the nature of the tests and are approximately correct for the circumstances. There is, however, convincing evidence that the lower values just cited are more realistic in terms of the over-all effects of aldosterone, such as in substitution therapy for adrenal insufficiency.

Other effects on electrolyte metabolism; life-maintaining action

The effects of aldosterone on electrolyte metabolism are not limited to the kidney. Mach *et al.* (68) noted that aldosterone depressed the salivary Na/K ratio but not sweat sodium. Pawan in collaboration with Simpson and Tait (97, 98) found in more extensive studies that the salivary Na/K ratio in normal human subjects could be depressed by minute quantities of aldosterone. A straight line dose-response relationship was obtained with doses of from 10–80 μg. Doses of 20 μg. were equipotent given either orally or intravenously. The depressed ratio was largely due to the decreased sodium content of saliva. Similar results were obtained in an Addisonian subject by Kekwick and Pawan (49). The urinary Na/K ratio of intact as well as adrenalectomized rats was depressed by aldosterone in acute experiments (98). Effective doses for normal animals lay between 0.1 and 0.5 μg.

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[3] In a subsequent personal communication they have indicated that the figure should be twenty to thirty times the activity of DC.

and after adrenalectomy this range was reduced to 0.01–0 05 μg. The response was not enhanced in either group by doses beyond the indicated ranges.

In adrenalectomized dogs, both Gross and Gysel (35) and Swingle *et al.* (111, 112) found that minute doses in the order of 1–2 μg./Kg./day would maintain life and electrolyte balance. Potency was in the range of ten to thirty times that of DC and five hundred times that of hydrocortisone. In both laboratories some qualitative differences from DC were noted. For instance, Swingle's group thought that the strong sodium-retaining effect of aldosterone, relative to its other weaker actions, was manifested by the fact that suboptimal doses maintained normal serum sodium levels and blood pressure. In a patient with Addison's disease receiving minimal effective doses, Kekwick and Pawan (49) noticed less effect on potassium and chloride balance than on sodium balance. All of these results tend to confirm the original observation (23) that the effect on potassium excretion is less marked than on sodium. Gross believed that aldosterone *overdosage*, however, caused less sodium and water retention than DCA (35).

In isolated rat diaphragms, Flückiger and Verzár (27) found that aldosterone and hydrocortisone affected permeability to electrolytes in a similar manner, but that aldosterone was more potent. The nature of this response was determined by the concurrent changes in carbohydrate metabolism.

A small amount of work has been performed with aldosterone in man, permitting some definite, but not comprehensive conclusions as to its actions. It is clear from the report of Mach *et al.* (68), followed by that of others (49, 86, 33, 116), that small doses will maintain electrolyte balance and well-being in Addisonian patients. Its potency would appear to be in the order of twenty to thirty times that of DCA, with the minimum human dosage of about 100 μg. per day. In the early experiments, water metabolism was not notably influenced (86, 33) and excess salt retention was not seen; small doses did, however, cause expansion of the extracellular space without augmentation of total body water (33). Later work in normal (116), rheumatoid (120) and Addisonian subjects (116) indicated that doses in the range of 1 mg. of aldosterone per day could cause some salt and water retention. Elevation of the plasma sodium level was seen in a pseudohermaphrodite (86). Thorn *et al.* (116) believe that the most sensitive indication of aldosterone activity in man is its depression of the Na/K ratio in urine, and that it is most effectively administered either intravenously or by frequent intramuscular injections in aqueous solution. Other effects of aldosterone in man are included under specific topic headings in this review.

Effects on water excretion

The effects of corticoids on water excretion are probably dependent on some interplay between the following factors: 1) their effect on sodium excretion, sodium retention being an antidiuretic influence and sodium loss a diuretic influence; 2) their effect on the glomerular filtration rate; and 3) their effects on the tubular reabsorption of water, which may be direct or conditioned by concurrent activity of the neurohypophysis. The result is that corticoids may be diuretic or antidiuretic depending upon: 1) the nature of the steroid used, 2) the dose, and 3) the physiologic or pathologic conditions under which they have to work. Clearly, the rate of water excretion is not solely dependent on the rate of sodium excretion (28, 23, 21).

Desaulles *et al.* (23, 21) have made a new comprehensive study of this problem in which the action of aldosterone was compared with that of many other steroids under the limited and well-defined conditions of their modification of the Kagawa test, in which rats are hydrated with isotonic sodium chloride solution. Aldosterone had no effect on water excretion at any dosage level, despite the fact that it caused sodium retention and potassium loss. DC, on the other hand, was antidiuretic in proportion to dose and in proportion to sodium retention. The effects of hydrocortisone, cortisone, and corticosterone, both on electrolyte and water excretion, differed among themselves and from the effects of either aldosterone or DC.

It is well known that adrenalectomized animals, or Addisonian patients cannot excrete a water load at a normal rate. This deficiency is poorly, if at all repaired by DC but readily repaired by cortisone or whole adrenocortical extract. In this respect, aldosterone resembles DC, as first shown by Gross (35). Doses of aldosterone as high as 1 mg. (34) did not restore water diuresis to normal in adrenalectomized dogs. The same result has been reported (although with smaller doses) in Addisonian patients (68, 86, 49, 33). In patients excreting large amounts of endogenously-produced aldosterone, no consistent relationship between urinary volume and urinary titers of the hormone could be found. An inverse relationship existed, however, between the amounts of aldosterone and sodium excreted (65).

These well-supported instances of the lack of effect of aldosterone on excretion do not, however, establish an invariable rule. In the course both of renal clearance and water intoxication studies in the authors' laboratory, aldosterone caused a distinct increase in water diuresis when given to adrenalectomized rats hydrated with distilled water *per os*. Its diuretic potency equalled that of hydrocortisone and exceeded that of cortisone and DC (87).

Glucocorticoid effects

a) *Stress.* Work performed in the authors' laboratory suggested that aldosterone was an active, or possibly more active than cortisone in protecting adrenalectomized rats against the stress of cold (30). It was certainly more potent than DC. In this generalized test, all known corticoids are effective in various dosages.

Soffer *et al.* reported that an Addisonian patient maintained on 100 μg. of aldosterone per day withstood the stress of an upper respiratory infection (temperature 103° F.). They believed that aldosterone provided better protection than would have been anticipated with any dose of DCA (33).

b) *Water intoxication.* Marked susceptibility to the stress of water intoxication is characteristically seen in adrenal insufficiency. This is due in part to an inability to excrete excess water and in part to a lack of resistance to the "toxic" effects of water as manifested by reduced body temperature, collapse, convulsions, hyponatremia and death. Equally effective protection against the experimental production of this syndrome in rats was afforded by aldosterone and hydrocortisone. In some respects, cortisone and corticosterone were probably weaker, and DC was clearly less effective.

c) *Carbohydrate metabolism.* Aldosterone caused a deposition of liver glycogen in adrenalectomized fasting mice (93). In this respect it showed about one-third the activity of cortisone, two-thirds the activity of corticosterone and thirty times the activity of DC.

The clinical literature suggests that its effects on blood sugar and glucose tolerance in man may be greater than anticipated from its DC-like actions (68, 49, 86). This has not been a consistent observation, however, and those who have made the most extensive studies have failed to see any effects of aldosterone on carbohydrate or protein metabolism (33, 120, 116).

d) *Eosinophil counts.* Studies in two laboratories, using the adrenalectomized mouse, indicated in one case that aldosterone was about one-half as active as cortisone (30) and in the other, about one-fourth (107). Since small amounts of material precluded precise work in both of these estimates, the suggested mean of one-third the activity of cortisone would be permissible and consistent with the results of other work.

Clinical studies indicate that aldosterone may reduce eosinophil levels in man (49, 116) but not consistently (68, 86, 33, 120).

e) *Allergy.* In the adrenalectomized guinea pig, sensitized to horse serum, aldosterone in doses of 100 to 500 μg. did not restore the protective influences of antihistamines (Pyribenzamine) against anaphylactic shock. In this respect, it resembles DC and differs from cortisone (34).

f) *Connective tissue: granuloma, inflammation.* Using the cotton pellet implantation technique, Desaulles, Schuler and Meier (22) found that aldosterone, unlike cortisone, when applied locally, will not inhibit the growth of granuloma tissue around a subcutaneous cotton pellet. At low dosage levels it may stimulate granuloma growth. Its over-all effects differed from those of any other corticoids.

Selye and Heuser (95) have reported that hydrocortisone inhibits the granuloma formation and exudation around subcutaneous pockets produced by injections of air and croton oil. This inhibition was blocked by small doses of DC but not by similar doses of aldosterone.

Mach and co-workers saw evidence of anti-inflammatory action in the first Addisonian patient treated with aldosterone (68). This has not been observed in specific studies on rheumatoid arthritis with doses up to 1 mg. per day (120).

g) *Renal clearances.* In adrenal insufficiency both the glomerular filtration rate and renal plasma flow are low and are better repaired by cortisone than by DC. The sodium-retaining action of aldosterone is manifested by doses which do not repair the low filtration rate (91). Nevertheless, Renzi *et al.* (87) found that the low filtration rate, renal plasma flow and water excretion of adrenalectomized rats can be elevated by aldosterone. In this respect the potency of aldosterone approximates that of hydrocortisone, which is itself more potent than cortisone and DC. The amounts of aldosterone required, however, are much greater than those necessary to inhibit sodium excretion. In Addisonians, slight elevation of endogenous creatinine clearance has been reported during aldosterone therapy (49).

h) *ACTH release.* Renzi, Gilman and Gaunt (88) found that aldosterone would suppress an expected stress-invoked release of ACTH. Its potency was about one-third that of cortisone and eight times that of DC. Relatively large doses of aldosterone (up to 1,050 μg./day) did not affect ACTH secretion in pseudohermaphroditic (86, 120), rheumatoid (120) or normal subjects (116), as judged by 17-ketosteroid and 17-hydroxycorticoid excretion.

Effects on blood pressure

Although aldosterone will maintain a normal blood pressure in adrenalectomized animals (112) and Addisonian patients (49, 86, 68, 33), no direct evidence yet exists that it will produce hypertension in the manner of DC and other corticoids. In fact, the absence of such effects has been noted (68).

The most extensive work on the subject is that of Gross *et al.* (36). These authors observed the effects of aldosterone under conditions in which DCA

is known to produce hypertension. They used adrenalectomized, unilaterally nephrectomized rats given a 1 per cent sodium chloride solution to drink. In these circumstances 1 mg. per day of DCA exerted its oft-noted effects: hypertension, vascular and renal lesions and a diabetes insipidus-like water exchange. When aldosterone was given in one twenty-fifth the dose of DCA—an amount chosen because aldosterone is about twenty-five times as potent as DCA in causing sodium retention—it showed none of the pathologic effects generally anticipated.

Miscellaneous effects

Meier and Bein have studied the effects of aldosterone on certain adrenal-dependent circulatory responses to epinephrine and histamine (79). Small quantities of aldosterone (10–30 μg. intravenously) kept the femoral blood-flow response to these drugs within normal limits in adrenalectomized cats. This effect could not be obtained with DC, cortisone, hydrocortisone, corticosterone, progesterone, ACTH or growth hormone. It is thus indicated that aldosterone may have unique effects on the peripheral vasculature. Further work in this field will be awaited with interest.

Loustalot and Meier (58) distinguished the effects of aldosterone from those of other corticoids on certain enzyme systems. In general, the qualitative effects of aldosterone resembled those of DC more than those of other corticoids.

Mach *et al.* (68) and Kekwick and Pawan (49) noticed a fading of pigmentation in Addisonian patients receiving small doses of aldosterone. The sensitivities of patients may differ considerably, since others have not noticed such effects (86, 33, 116).

Aldosterone has failed to inhibit the response of the isolated frog heart to acetylcholine and in this respect differs from both the corticoids and sex steroids (34).

Adrenal steroids are necessary for the initiation and maintenance of lactation in adrenalectomized rats and guinea pigs. In the rat, 50 μg. per day of aldosterone had about the same lactation-maintaining effect as 1 mg. of hydrocortisone (19).

ALDOSTERONE IN URINE

In 1950 Deming and Luetscher (20) reported that a sodium-retaining substance, resembling DC in its biologic properties, was present in the urine of patients with edema and low urinary sodium excretion associated with cardiac or renal disease. In a long series of continuing studies, the group under Luetscher's leadership at Stanford gradually characterized this substance both chemically and biologically (64, 62, 61, 63, 60, 3, 66,

65). Recently it has been identified unequivocally as aldosterone (67).[4]

While this work was in progress, an intensive and productive study of aldosterone-like sodium-retaining factors (SRF) in urine was being conducted by Gordon, Shipley and Chart at the University of Wisconsin (13, 12, 32) and Venning, Singer and associates at McGill University (119, 118, 8, 78, 106). Lloyd (56) observed DC-like substances in eclamptic urine. Interestingly enough, the excretion of Lloyd's "poorly water soluble" corticoid was apparently inhibited by DCA (*cf.* 104).

In general, excellent agreement was obtained by the various groups concerning the circumstances which affect the secretion of SRF. It may be present in excess amounts in nephrosis (20, 63, 65, 67, 78), heart failure (20, 65, 32, 106, 66, 18), eclampsia (13, 32, 119, 118) and hepatic cirrhosis (61, 65, 12, 32, 84). Using a sensitive intravenous bioassay method, Lobotsky *et al.* (57) have reported preliminary evidence suggesting that the plasma levels of SRF may be increased to twenty-five times normal in cirrhotic patients.

If adequate methods of extraction and assay are used, detectable amounts of SRF are found in normal urine (12, 32, 62, 18, 55) but the quantities are such that errors of assay may be easily misleading (65).

An enhanced excretion of SRF has been reported in cases of malignant, but not of essential hypertension (118). It was not observed in the urine of 4 patients with rheumatoid arthritis under conditions in which it was detectable in 4 of 6 normal subjects (18). Llaurado (55) found SRF titers increased postoperatively, and associated that fact with the characteristic renal loss of potassium which follows surgical operations. His results are consistent with those of the post-surgical metabolic studies of Moore *et al.* (81).

The presence of edema or of one of the aforementioned underlying diseases does not in itself assure that there will be a high level of aldosterone-like material in the urine. Luetscher (65) thinks that one reliable index which permits prediction of a high aldosterone excretion is that of a low sodium excretion (10 mEq./day or less).

The adrenal origin of the material is indicated by the fact that little or none is present in the urine of Addisonian or adrenalectomized patients (32, 62).

The question of paramount interest concerns the cause-and-effect relationship of aldosterone to the various diseases in which it is excreted in increased amounts. Although there is some suggestive evidence that aldo-

[4] In recent publications, Neher and Wettstein (82) have described physico-chemical methods for the extraction and measurement of aldosterone in body fluids and tissues; Clark (14) has provided data on the absorption spectrum and color reactions of the compound; and Pechet (83) and Schmidt and Staudinger (92) have described methods for its chromatographic isolation.

sterone administration may cause excess sodium and water retention (42. 124), edema was not present in some patients spontaneously excreting large amounts of aldosterone-like substance from known or presumed adrenal tumors, as discussed subsequently (18, 16, 70). It may be that aldosterone causes edema (a) if it is present in excess, and (b) if in the presence of other as yet undefined physiologic or pathologic circumstances. "It is important to recognize that many of these changes in renal and endocrine function are the result, not the cause, of the disease" (59).

HYPERALDOSTERONISM

Conn has recently described a case interpreted to represent a syndrome of *primary aldosteronism* (16). Mader and Iseri (69, 70) reported an almost identical case. Subsequent surgery demonstrated the presence of adrenal tumors in both instances (17, 70). The syndrome was characterized by high titers of aldosterone-like materials in the urine, hypokaliemia, hypernatremia, alkalosis, polyuria (Pitressin-resistant in Conn's case), intermittent tetany, muscular weakness, and hypertension. There was no significant edema in either case. There was, however, some refractoriness to the usual effects of potassium administration. 17-Hydroxycorticoid excretion was either normal (16) or only slightly elevated (70). Mader and Iseri emphasized the marked hypomagnesemia and negative magnesium balance in their case. The significance of this interesting observation remains to be determined. They also noted adrenal atrophy, suggesting that pituitary-suppressing steroids were being elaborated. Renal damage, although present, was thought to be eliminated as a primary etiologic factor. Conn suggests that other examples of "potassium-losing nephritis" are instances of the same syndrome (16, 15). High urinary levels of SRF have been reported in one such case (18).

It is of great interest that some adrenal tumors probably exist which secrete excess aldosterone. These serve as counterparts of those tumors which produce primarily 17-hydroxycorticoids and cause Cushing's syndrome and those which produce androgens and cause the adrenogenital syndrome. These cases also perhaps define true chronic aldosterone overdosage effects in a way not yet possible experimentally because of the scarcity of the hormone. The syndrome of *primary aldosteronism* perhaps differs from other conditions in which aldosterone excretion is excessive—hepatic cirrhosis, or nephrotic syndrome—because the increased hormone production may be a primary cause in the one instance and an effect in the others.

ALDOSTERONE IN BLOOD

Simpson and Tait detected a substance in adrenal venous blood which, in their bioassay procedure, behaved like aldosterone (99). Subsequent

chemical fractionation studies of systemic blood left little doubt of its identity and it was estimated that normal human systemic blood contains approximately 0.08 μg. per 100 ml. (97, 98). Wettstein (123) reported that beef blood contains 0.35 μg. per 100 ml. This estimate led to the calculation that the amount circulating in beef blood is about seventy times greater than that in adrenal tissue. Farrell *et al.* (26) definitely identified aldosterone in the adrenal vein blood of hypophysectomized dogs. The presence or absence of ACTH had little effect on the rate of aldosterone secretion, although it had its expected effects on the section of other corticoids. Singer and Stack-Dunne (104) have published data in rats concerning the blood levels of aldosterone expressed as DC equivalents in a variety of conditions.

Unger *et al.* (117) found an aldosterone-like material in perfusates of calf adrenal. Judging by bioassay, the calf gland produced 0.3 μg. per Gm. per hour.

Determinations of blood concentrations in various conditions are of crucial importance in assessing the significance of the new work with crystalline aldosterone. It is probable, if the foregoing estimates are correct, that some of the "glucocorticoid" effects of injected aldosterone reviewed in earlier sections are overdosage effects; the hormone under normal conditions may never reach the concentration necessary for these effects to be manifested. The pharmacology of overdosage with steroids such as aldosterone is a subject of great importance, but it is well for the distinction between pharmacologic and physiologic doses to be recognized.

ALDOSTERONE IN PLACENTAL EXTRACTS

Gordon *et al.* (32) found sodium-retaining activity in placental extracts but doubted that amounts were sufficient to implicate the placenta as a source of the excess SRF in the urine during toxemia of pregnancy. Majnarich and Dillon (71) have presented suggestive evidence from chromatographic studies for the presence of aldosterone in placental extracts.

METABOLISM OF ALDOSTERONE

No comprehensive studies are yet available concerning the metabolism of aldosterone. In 1951 Bongiovanni and Eisenmenger (5) pointed out that the altered pattern of steroid metabolism and electrolyte balance in liver disease suggested that the liver disease was associated with an increased production of sodium-retaining steroid. More specifically, the Wisconsin group (10, 31) reported that the SRF of urine is inactivated by the liver. Presumably aldosterone is excreted in some conjugated form. It is generally agreed that yields from urinary sources are increased by acid hydroly-

sis (82). β-Glucuronidase hydrolysis increased yields somewhat, but less than strong acid (32, 119, 2). When crystalline aldosterone is injected, only traces are found in the urine (123, 80). An anomalous increase has been noted in formaldehydogenic steroid excretion in Addisonian patients after the injection of crystalline aldosterone; the amounts excreted could not have been accounted for by conversion of the injected steroid (33). This finding is not entirely consistent with that of other workers who have failed to find distinct effects of aldosterone on either 17-ketosteroid or 17-hydroxycorticoid excretion (68, 86, 120, 116). The possible beneficial effect of adrenalectomy in hepatic cirrhosis with ascites, and its relation to the role of aldosterone have been discussed (73).

CONTROL OF ALDOSTERONE SECRETION

Effects of hypophysectomy and ACTH

Although the mechanisms which regulate the secretion of aldosterone are not yet known, one important fact is all but certain: aldosterone is not under the complete and direct control of ACTH, as are the other major corticoids.

It has long been apparent from the fact that life and electrolyte balance can be maintained after hypophysectomy and that hypophysectomized animals die promptly after adrenalectomy, that the adrenal must secrete some DC-like material independent of the pituitary. This fact was perhaps first prominently discussed by Swann (109), although it has long been regarded by endocrinologists as self-evident.

Several investigators have found that ACTH administration in man does not significantly increase the blood (98) or urine (3, 32, 118, 18) levels of aldosterone-like materials under conditions which cause an increased production of other corticoids. In Cushing's syndrome one group finds SRF increased (118), whereas another finds it unaffected (32). Simpson and Tait thought that blood titers in their cases may have been reduced by ACTH (98). It may be pertinent to recall that ACTH therapy is beneficial in the nephrotic syndrome and may result in reduced aldosterone excretion (63, 126); the means by which ACTH reduces aldosterone output, however, may be indirect.

Farrell *et al.* (26) found that after hypophysectomy in the dog, aldosterone secretion continued in measurable amounts—not enhanced by ACTH. In fact, aldosterone was the only corticoid present after hypophysectomy in amounts sufficient to exert important metabolic effects. This is entirely consistent with the observation that panhypopituitary patients excrete normal amounts of aldosterone (32, 62), although the secretion of other corticoids is greatly reduced.

In the rat the situation may be different. Singer and Stack-Dunne (104) found the rate of secretion of aldosterone-like steroid, as measured in adrenal vein blood, to be reduced by hypophysectomy from the rate observed in intact animals under stress. ACTH restored both aldosterone and corticosterone (the major corticoid produced by the rat adrenal) secretion rates essentially to normal after hypophysectomy. The administration of DCA or a reduction in potassium intake markedly depressed aldosterone but not corticosterone secretion.

Results reported from Venning's laboratory (118, 8) suggest that the excretion of aldosterone-like substances is increased in man by injections of pituitary growth hormone. This observation, if confirmed, has intriguing possibilities, since aldosterone is obviously not completely under control of ACTH. Growth hormone itself can cause sodium retention in hypophysectomized animals (4), an effect conceivably exerted through the adrenals. Unlike aldosterone, however, growth hormone also causes potassium retention, and its electrolyte-retaining effects may be incidental to its growth-promoting properties. Even if growth hormone could be demonstrated unequivocally to stimulate aldosterone secretion, the problem would still not be solved; the fact remains that aldosterone is secreted in the complete absence of the pituitary.

Effects of electrolyte intake

Another factor which seems to be involved directly or indirectly in modifying aldosterone secretion is that of electrolyte intake. Aldosterone excretion is enhanced by a low sodium diet (3, 62) and in rats its secretion is inhibited by low potassium diets or by DCA (104). Laragh and Stoerk (54) have provided strong evidence in the dog, supported by observations in man and the rat, that an increase in the plasma potassium ion or a decrease in the plasma Na/K ratio may be the most potent stimulus to aldosterone secretion. It is not known, however, whether this stimulus is direct or indirect.

The available data do not permit comprehensive conclusions as to the regulation of aldosterone secretion. It is clear, first, that it is at least in part independent of the pituitary, but perhaps not completely so. It is also clear that its rate of secretion is in part regulated, either directly or indirectly, by electrolyte balance.

SUMMARY AND CONCLUSIONS

Aldosterone, the long-sought active principle of the "amorphous fraction" of adrenal extract, has been crystallized, its formula established and its true nature as a hormone proved by its isolation from adrenal and systemic vein blood and from urine. It has not been established unequivo-

SUMMARY OF BIOLOGIC ACTIVITY OF CRYSTALLINE ALDOSTERONE

Test	Subject	Activity	Reference No.
Na retention	Adx. rat	20–30×DC	23, 47, 11
K excretion	Adx. rat	5×DC	23
Na^{24}/K^{42} excretion	Adx. rat	120×DC	107
Na/K excretion	Adx. rat	30–100×DC	77, 26, 90
Water (saline) excretion	Adx. rat	No retention, unlike DC; no diuresis, unlike cortisone	23, 21
Water diuresis	Adx. dogs; addisonians	Weak or inactive, like DC	35, 34, 68, 86, 49
	Adx. rats	Active: >DC; >cortisone; =hydrocortisone	87
Water intoxication	Adx. rats	>DC; probably >cortisone and corticosterone; =hydrocortisone	87
Renal functions: rise of GFR & RPF	Adx. rats	>DC; >cortisone; =hydrocortisone	87
Renal functions: Na retention *vs.* GFR	Adx. rats	Na retention without elevation of GFR	91
Substitution therapy	Adx. dogs	12–25×DC; 500×hydrocortisone	35, 112
Substitution therapy	Addisonians	Maintained with 100–200 μg./day; 20–30×DC	68, 49, 86, 33
Electrolyte balance	Man	Caused Na and water retention; weight gain	120, 116
Na/K of saliva	Man	Depressed by10–20 μg.	98
Reduced permeability to Na and K	Rat diaphragm	>10×hydrocortisone	27
Cold stress	Adx. rat	= or >cortisone	30
Reduction of eosinophil count	Adx. mouse	⅛ cortisone	30, 107
Liver glycogen	Adx. mouse	⅛ cortisone; 30×DC	93
ACTH suppression	Rat	⅛ cortisone; 8×DC	88
Antagonism to allergic shock	Adx. guinea pig	Weak or inactive, like DC	34
Granuloma growth—cotton pellet	Adx. rat	Inactive or stimulatory, unlike other corticoids	22
Granuloma pouch—air pocket	Adx. rat	Inhibited cortisone less than DC	95
Blood pressure	Adx. rat	Maintains B.P. in adrenal insufficiency; not hypertensive	36
Blood flow in femoral arteries	Adx. cat	Maintains femoral flow response to epinephrine & histamine, unlike other corticoids	79
Inhibition of acetylcholine	Isolated frog heart	Inactive, unlike other adrenal & sex steroids	34
Enzyme systems	Rat	Slight differences compared with DC & other corticoids	58
Maintenance of lactation	Adx. rat	20×hydrocortisone	19

References concerning the effects of crystalline aldosterone in man: 98, 49, 68, 120, 86, 33, 116.

cally that all sodium-retaining activity in adrenal extracts or body fluids is due to aldosterone or other known steroids. No evidence exists, however, which contradicts that hypothesis. An economic method of synthesizing aldosterone is sorely needed because there is no known natural source which is adequate to supply sufficient hormone for research or therapeutic purposes.

The limited, although highly revealing, work performed to date is too restricted to permit full definition of the physiologic role of the hormone or its pharmacologic properties. Certain tentative conclusions can, however, be drawn. It is probably the main endocrine agent regulating sodium, potassium and chloride metabolism; it is perhaps also involved in magnesium metabolism. Although it is the most active mineralocorticoid known, its actions are not limited to effects on electrolyte metabolism. In appropriate dosage, its effects can mimic those of hyrocortisone and it has some unique properties of its own. It is not just a more potent desoxycorticosterone; important qualitative as well as quantitative differences are clearly suggested between DC and aldosterone. In particular, no evidence yet exists that the administration of aldosterone causes the pathologic overdosage effects of DC when the two steroids are given in doses that are equipotent from the standpoint of sodium retention. no hypertension

The human diseases in which aldosterone may possibly be incriminated as a causative factor are those in which high rates of aldosterone excretion are associated with sodium retention and edema (*e.g.*, congestive failure, or nephrosis) and in the less well-known states of "primary aldosteronism" and "potassium-losing nephritis." In the diseases characterized by edema, it is easier to visualize excess aldosterone as being involved only in certain acute phases than as an etiologic agent.

An understanding of the physiology of the hormone in health and disease must await elucidation of the mechanisms by which its rate of secretion is regulated. The clear facts in that regard are: a) that its secretion continues in the absence of the pituitary (as early theory proposed); b) that ACTH in man and the dog, but perhaps not in the rat, does not affect its rate of secretion; and c) that directly or indirectly, its rate of secretion is affected by electrolyte intake. In addition and less certainly, it is possible that growth hormone stimulates its output, and that other corticoids suppress its secretion.

The observation that low-salt diets call forth a compensatory secretion of aldosterone means that the effectiveness of such diets is somewhat self-limited. For this and other reasons, the discovery of some practical means by which aldosterone secretion may be inhibited would provide a welcome therapeutic tool.

Addendum

Conn (*J. Lab. & Clin. Med.*—in press) has provided follow-up data on his case of *primary aldosteronism* (16). An adrenal was removed containing a cortical adenoma which apparently secreted excess amounts of aldosterone. The operation resulted in almost complete elimination of the metabolic abnormalities. Postoperative injection of 1 mg. of aldosterone per day for three days changed most biochemical indices in the direction of the original abnormality. The histologic status of the gland contralateral to the tumor was interpreted to indicate that the zona fasciculata is the site of aldosterone secretion. Conn quotes evidence from others indicating the probable existence of 5 additional patients with this syndrome, 4 of whom had cortical adenomas although 1 definitely did not.

Luetscher and Curtis (*Proc. Am. Soc. Clin. Invest.*, 1955, in press) report a high rate of aldosterone excretion in a case with "salt-losing nephritis." Presumably, therefore, this condition is due to an inability of the kidney to respond to aldosterone-like steroids, rather than to a hormonal deficiency. In normal men, however, Luetscher and Axelrad (*Proc. Soc. Exper. Biol. & Med.* **87:** 650, 1955) confirmed the observation that a low-sodium diet caused a high aldosterone excretion which mirrored the reduction of urinary sodium. The low-sodium diet did not affect renal clearances or the excretion of other types of corticoids.

Selye has reported (*Science* **121:** 368, 1955) that larger doses of aldosterone than he used originally (95) blocked the anti-inflammatory actions of hydrocortisone. The potency of aldosterone in this respect was apparently roughly equivalent to that of DC. The significance of such results, like that of others mentioned previously, will become more clear when and if it is established that enough aldosterone is normally secreted to antagonize some of the actions of endogenous hydrocortisone under physiologic or defined pathologic conditions. Desaulles *et al.* (personal communication) have, however, made the related observation that aldosterone can block to some extent the sodium-excreting effect of cortisone in rats.

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Editorial

ON MORPHOGENIC CAPACITIES OF THE ESTROGENS*

THOUGH actually there exist no separate metabolic and morphogenic functions of a hormone, usually it is expedient to present chemistry and morphology as independent aspects, simply because their operational relationship still is obscure. The most important recent progress in the interpretation of hormone action stems from the realization that it is involved in the enzymatic control of cytochemical processes (1). Hormones being without effect on the course of reactions between pure chemicals, or in homogenates, it may further be concluded that more directly they affect some structural elements of the organismic constitution, thereby producing changes of permeability, surface tension, and other physico-chemical conditions (2, 3). Of particular interest is the fact that many enzyme reactions reveal a pre-existing distribution pattern in the organism, which they only bring to light but do not create. The classic example was furnished by Fishman and Fishman (4), who discovered the selective increase of β-glucuronidase activity in the tissues of secondary sex characters after estrogen administration, and in non-sexual tissues after administration of menthol and other glucuronidogenic drugs (5). It will be remembered that glucuronidase routinely is measured by its *hydrolyzing capacity* in the homogenized tissues. In the living organism it catalyzes the synthesis of glucuronides. The prevailing direction of these reactions depends on concentration conditions. Fishman condenses his observations into a hypothesis of *adaptive response of the synthetic activity of β-glucuronidase to substrates* (6). The growth theory of Levvy, Kerr and Campbell (7) extends one step further, in connecting glucuronidase activity with the rate of mitotic cell division. Both concepts are of interest to the embryologist who tries to evaluate the role of hormones in the morphologic determination and differentiation of embryonic tissues. Foote (8) has described remarkable effects that estrogens produce on the development of the oviducts. Normally, first the funnels of the oviducts appear in the form of depressions in the peritoneum, and then the tubular parts grow from the blastemic pits

* Dedicated to Dr. Alfred Kühn, Director of the Max Planck Institute for Experimental Biology at Tübingen, in honor of his seventieth birthday.

downward along the nephric bodies until they reach the cloaca. If estrogens (estrone, estradiol) are administered during this period, the diameter of the tubes enlarges and the general histologic structure prematurely assumes the adult character. At the same time, however, the downgrowth of the tubes becomes arrested; the blastemic lower tips are used up in the process of *adultization*. Thus, only a thickened upper part of each oviduct is formed, its length expressing the time of onset of estrogenic action. If this action intervenes before the beginning of oviduct differentiation, the capacity of the particular region of the peritoneum (the preprimordium) to produce an oviduct is abolished. Of the two features of *adultization:* functional growth of the primordium, and suppression of primary differentiation, only the latter is realized if the hormone is administered at an early stage. This effacement of pre-existing embryonic determination in the funnel fields as well as in the growing tips of oviducts definitely rules out the possibility that in the normal, genetically determined development, estradiol or any other estrogen might be the inductive substance for oviduct formation. The observations of Foote find a striking parallel in Raynaud's work on mammary development in the mouse (9).

The deterioration of embryonic preprimordia exposed to estradiol calls for a consideration of other destructive actions of this hormone. First among them stands the observation by von Moellendorff (10) that in fibroblast cultures estradiol is a toxin that delays mitosis and causes fragmentation and loss of chromosomes. Knake (11) was inclined to consider this as indirect evidence in support of the notion that in the normal body the active estrogenic principle is not the simple hormone, but some of its conjugates. Similar suggestions are found also elsewhere in the literature, but they seem to disagree with observations reported by Wolff (12) on normal reactions to estrogens by sexual tissues cultured *in vitro* for several days.

Moreover, the toxic effects are not restricted to cultured fibroblast cells, but appear likewise in cleaving amphibian eggs immersed in weak solutions of estradiol in water (about 1 to 10 μg. per liter). Töndury (13) working with Triton eggs obtained very irregular morulae and blastulae, and the histologic study disclosed again a destructive effect on chromosomes and mitotic spindles. These germs die before gastrulation. However, even earlier, Waddington and Needham (14) had announced seemingly contrary results. They implanted very tiny pieces of coagulated egg albumen that contained estrogens, into the ventral blastocele of early gastrulae. As a result of such local application, secondary ventral embryo formation was induced. The net effect was an induction of differentiation. The concentration of the estradiol in the albumen was about 1,000 times that of the water solution in Töndury's experiment, but since the hormone had to reach the living cells by diffusion, the actual densities at the place of reac-

tion were probably of similar order. It appears now most probable that in the second experiment the estrogen was not acting directly by substituting for the natural inductor substance, but that it merely unmasked inactive inductor, which is present in all cells of amphibian blastulae. Its immediate effect was the same in both experiments, namely, cellular degradation. This process, it is well known, tends to liberate the inductive principle (15).

The toxic effects of estradiol are not restricted to fibroblasts and cleavage cells, but appear also at much later stages, when there are well developed circulatory systems. Töndury (16) showed that teratologic development starts usually in blastemas with high mitotic activity, such as eyeballs of hatching larvae, and limb buds. He also pointed out that these are tissues with high concentrations of ribonucleic acid. Töndury's experiments on early and advanced stages of salamanders were confirmed in our laboratory with similar work on frogs (Tahmisian and Chang, unpubl.). Together with the data of Waddington and Needham, they seem to contain the clue for an understanding of the spectacular and complex morphogenic actions of estrogens, including the partial or total reversal of the sexual differentiation of the gonads, particularly in amphibians and in birds.

The reactions may differ in detail from species to species, but they fall clearly in two classes: *feminization* and *masculinization*. It should be remembered that the still undifferentiated gonads of genetically (chromosomally) male and female embryos and larvae consist of double primordia, *cortex* and *medulla*. In normal ovarian differentiation the cortex takes the lead on the basis of a genic balance that favors the female sex. Conversely a genic balance with male epistasis favors the medullary component, which leads to testicular differentiation. It has long been recognized that cortex and medulla are, in fact, a pair of competing and antagonistic inductors (18). Experimentally introduced estrogens are often capable of upsetting the genetically adjusted system. True to the role that they play in the aforementioned inductive processes, here again the estrogens act negatively, suppressing or eventually fully effacing the inductive capacity of either the medulla or the cortex. As the two are antagonists, the weakening of the genetically epistatic inductor permits the hypostatic partner to assume control and bring about a reversal, contrary to genetic determination (17).

The most perplexing feature of this interference of estradiol with normal sex differentiation is the fact that it may be either feminizing or masculinizing in the end effect, depending on species characteristics, and on hormone concentration. In feminizing, estradiol depresses the heterotypic, *i.e.*, the medullary inductor, whereas in the case of masculinization it causes the reduction and almost complete disappearance of the homotypic, *i.e.*,

the cortical inductor. Without exception, feminization is obtained with lower hormone concentrations than is masculinization, but the level at which the effect is reversed varies from one species to another. In the European frog, *Rana temporaria*, it lies near 250 μg. per liter, whereas in the American *R. sylvatica* it is at 1,000 μg. per liter—near the limit of solubility of estradiol in water. Since, as a rule, the introduction of a hormone into an animal tends to depress the activity of the homotypic endocrine gland, one might consider masculinization as the more readily understandable and even as the expected reaction. However, at this stage the cortex is not an estrogen-producing gland and consequently the rules derived from observations on adult endocrine organs do not necessarily apply. So far, no species has been found in which even the lowest efficient dose of estradiol would produce masculinization. On the other hand, in salamanders, testosterone feminizes the genetical males at all levels (19, 20). Histologic study proves that in this case both inductors are greatly affected; but whereas the cortex is only reduced to a rudiment that later may recover, the medulla often fails even to start differentiating (19). Therefore, this salamander case presents a perfect parallel to the deletion of the preprimordia of oviducts and of mammary glands by estradiol.

All attempts at analyzing the nature of hormone actions thus lead to the recognition of pre-existing patterns that determine the special quality of the evoked responses. Such latent patterns may be conceived as set-up reactions, ready to run their course, but shackled by some physico-chemical factor. It seems logical to assume that in an evolutionary sense the basic reaction is older and that its hormonic control represents a later addition. If so, "shackles" and hormones should have arisen simultaneously, one as the complement of the other. Hormonal structure as conceived by Schueler (21) would have to be matched by that of the inhibitor.

Evidently, the localized patterns, preprimordia and primordia in the embryologic sense, become organized on the basis of the hereditary constitution of a developing germ. Significantly, the geneticist today is as much concerned about problems of pre-existing patterns as is the endocrinologist. As it is known that many cytochemical enzyme reactions are gene controlled, it even appears conceivable that the chromosomes may release substances that act in a manner similar to that of the true hormones.

In attempting the interpretation of hormonal sex reversal and its relationship to hereditary sex determination, one cannot fail also to probe into the possibility, that the genic reaction chain itself may result in the production of a steroidal gene-effector. However, one should not forget that sex reversal can be brought about by a great variety of experimental methods, surgical, physical, chemical and biologic. Their analysis reveals as the common effective factor the reduction or elimination of one or the other in-

ductor, cortex or medulla. On the other hand, no facts are known that could support the contention that extra estrogen is produced by the cortex at or before the inception of sex differentiation. Experimental sex reversal always can be traced to *selective inhibition* of one or the other component of the gonad, whereas genic sex differentiation proceeds from an *activation and rapid enlargement* of either cortex or medulla. Moreover, a large body of facts supports the notion that the gene-determined inductor substances are of protein nature. However, the possibility is left open that the antagonism between cortex and medulla may be mediated by some steroid proteins (17).

In summary, the discussion of experimental facts now available leads to the conclusion that the morphogenic capacities of hormones, and in particular of the estrogens, result from the breakdown of complementary inhibitors of set-up reactions. This negative type of action sometimes assumes a destructive "toxic" character and under special conditions of timing and concentration may efface, rather than develop, latent preprimordial patterns.

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Letters to the Editor

EFFECT OF TEMPERATURE ON HYDROLYSIS OF 17-KETOSTEROID CONJUGATES IN URINE

TO THE EDITOR:

In his Letter to the Editor (*J. Clin. Endocrinol. & Metab.* **13**: 1544 (Dec.) 1953) Dr. Pond stated that he could not confirm the results reported by Jensen and Tötterman in 1952 and that the reason for this discrepancy was not clear.

Dr. Pond, who tried to reproduce the results on the basis of a preliminary description of the method given in *Nature* **169**: 374 (March) 1952, boiled the urine. However, we did not say that it should be boiled, but heated to 100° C. This explains the discrepancy.

In a later paper (*Acta endocrinol.* **10**: 33 (Sept.) 1952) we published a detailed report of the method, in which we stated that the urine should be heated *in a water bath at* 100° *C.*

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PHYSIOLOGIC ACTIVITY OF TRIIODOTHYROACETIC ACID

TO THE EDITOR:

The recent report by Thibault and Pitt-Rivers (1) on the effectiveness of the acetic-acid analogues of thyroxine and triiodothyronine in raising oxygen consumption of rat kidney slices prompts us to report our experience in the treatment of human myxedema with the latter compound. The results reported by Thibault and Pitt-Rivers are unique in two respects: 1. The acetic-acid analogues of thyroxine and triiodothyronine (tetraiodothyroacetic acid and triiodothyroacetic acid) raised the oxygen consumption of rat kidney slices without a preincubation period. 2. The maximum effect was reached in fifteen minutes and disappeared by ninety minutes.

They also reported that a single dose of tetraiodothyroacetic acid administered to thyroidectomized rats raised the basal metabolic rate within

two hours and the effect wore off after four hours. On the other hand, in a single experiment, a dose twice as great as the effective dose failed to raise the metabolic rate of the animal.

We have tested the activity of triiodothyroacetic acid in 2 patients with ordinary myxedema. In both, the material was administered daily intravenously.

In the first case the initial dose of 0.1 milligram was gradually increased to 1.0 milligram over a period of sixteen days. There was little or no detectable change in the level of the basal metabolic rate (−27 per cent), but the patient lost 3 kilograms in weight, the blood cholesterol concentration was reduced from 507 mg. to 400 mg. per 100 ml., the protein-bound iodine concentration rose from 0.7 μg. to 4.4 μg. per 100 ml., and the patient felt and looked better.

In the second case the initial dose of 0.4 milligram was progressively increased to 4 milligrams over a period of seventeen days. Again there was little or no rise in the level of the basal metabolic rate (−40 per cent), but the weight was reduced by 3 kilograms, the blood cholesterol concentration dropped from 533 mg. to 258 mg. per 100 ml., the protein-bound iodine concentration rose from 0.5 μg. to 11.5 μg. per 100 ml., and the patient also looked and felt better.

The rapid action of triiodothyroacetic acid on the oxygen consumption *in vitro* and of tetraiodothyroacetic acid *in vivo* may explain our failure to observe an increase in the basal metabolic rate of our patients, inasmuch as the material was administered in the afternoon and the basal metabolic rate was obtained the following morning. However, our results are not entirely consistent with those reported earlier by Pitt-Rivers (2), who found that these analogues had considerable potency in the prevention of thiouracil goiters of rats when administered daily for nine days, and that triiodothyroacetic acid showed considerable activity in increasing the metabolic rate of intact rats when administered daily for five days. The latter compound, according to Bruce, Pitt-Rivers, and Sloviter (3), was also capable of replacing thyroxine in the induction of the characteristic changes in the feathers of thyroid-deficient birds.

On the basis of the *in vitro* results, Thibault and Pitt-Rivers suggested that the acetic-acid derivatives of thyroxine and triiodothyronine are indeed the forms in which the thyroid hormone acts in tissue. Whether or not this suggestion is valid cannot be decided at present. Many more experiments are necessary to reconcile the *in vitro* and *in vivo* results, as well as the results in intact animals versus those in thyroidectomized animals and in patients with myxedema.

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The Endocrine Society

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3 and 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

(For program, *see* April issue of the Journal.)

THE 1955 AWARDS AND FELLOWSHIPS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than November 1 each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by November 1 each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE SEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE INDIANA UNIVERSITY SCHOOL OF MEDICINE AT THE CONTINUATION STUDY CENTER OF THE INDIANA UNIVERSITY MEDICAL CENTER, INDIANAPOLIS, INDIANA

September 26–October 1, 1955

The faculty will consist of twenty-one eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program, write Post Graduate Office, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis 7, Indiana. Registration is limited to 100; tuition fee is $100.00. Arrangements for housing accommodations will be made through the Indiana Medical Center.

BOOKS RECEIVED

Blutkrankheiten. Methodik, Klinik und Therapie. By PROF. DR. HANNS FLEISCHHACKER. Sections on methods for study of the blood plasma; the morphologic elements of the blood and their reactions; relationships of elements involved in coagulation; the bone marrow; the pathology of erythropoiesis, leukopoiesis, thrombopoiesis; the hemorrhagic diatheses; the pathology of the reticulum cells and the plasma cells; and a summary of blood and bone-marrow changes in the common infectious diseases; 240 pages; 76 illustrations (many in color); 1955. Vienna, Wilhelm Maudrich. Sole representative in U.S.A. and Canada, Intercontinental Medical Book Corp., New York, N. Y. Price $7.50.

Brookhaven Symposia in Biology, No. 7. The Thyroid. Several authorities in the field collate and discuss findings and ideas concerning problems of thyroid and thyroid hormone physiology presented at the Annual Symposium held June 9 to 11, 1954, Brookhaven National Laboratory, Biology Department, Associated Universities, Inc., under contract with the United States Atomic Energy Commission, Upton, Long Island, New York; 278 pages; illustrated; 1955. Available for distribution to persons and organizations associated with the national atomic energy program. Available to others from the Office of Technical Services, Department of Commerce, Washington 25, D. C. Price $1.75.

Differential Diagnosis of Internal Diseases. Clinical Analysis and Synthesis of Symptoms and Signs on Pathophysiologic Basis. Second revised and enlarged edition. By JULIUS BAUER, M.D., Clinical Professor of Medicine, College of Medical Evangelists; Senior Attending Physician, Los Angeles County General Hospital; Los Angeles, California; in two parts—I. Leading Symptoms, and II. Leading Signs; 1002 pages; 66 illustrations; 2 tables; 1955. New York, Grune & Stratton, Inc. Price $15.00.

Ion Exchange and Adsorption Agents in Medicine. The Concept of Intestinal Bionomics. By GUSTAV J. MARTIN, Sc.D., Research Director, The National Drug Company, Philadelphia, Pa.; 340 pages; 15 line drawings and 11 photographs; 49 tables; 1955. Boston, Mass.; Little, Brown and Company. Price $7.50.

Letalfaktoren in ihrer Bedeutung für Erbpathologie und Genphysiologie der Entwicklung. By PROF. DR. ERNST HADORN, Professor of Zoology and Comparative Anatomy at the University of Zurich. A monograph on the hereditary factor that brings about death at a certain stage of the existence of the individual organism, and the relationship to modern genetics and developmental physiology and to mutation prophylaxis; 338 pages; 129 illustrations. Stuttgart, Georg Thieme Verlag. In U.S.A. and Canada, Intercontinental Medical Book Corp., New York, N. Y. Price DM 39. $9.30.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Sales Office, P.O. Box 575, Packanack Lake, New Jersey.

Cold Injury. Third Conference, February 22, 23, 24 and 25, 1954, Fort Churchill, Manitoba, Canada. Edited by M. IRENÉ FERRER, M.D., Assistant Professor of Clinical Medicine, Columbia University College of Physicians and Surgeons, New York, N. Y.; 226 pages; 31 figures; 47 tables; 1955. Price $4.50.

Gestation. First Conference, March 9, 10 and 11, 1954, Princeton, N. J. Edited by LOUIS B. FLEXNER, M.D., Professor and Chairman, Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, Pa.; 238 pages; 97 figures (19 in color); 11 tables; 1955. Price $5.00.